A — mRBD, B — iRBD, C — mSpike, D — iSpike: OD (490 nm) versus Reciprocal dilution. Legend: post NL63, other control samples, SARS-CoV-2 #1, SARS-CoV-2 #2, SARS-CoV-2 #3A, SARS-CoV-2 #3B.

E — mRBD, F — iRBD, G — mSpike, H — iSpike: Area under curve (AUC) for Control samples versus SARS-CoV-2 seroconverters; $p < 0.001$.

A

mSpike isotyping

B

iSpike isotyping
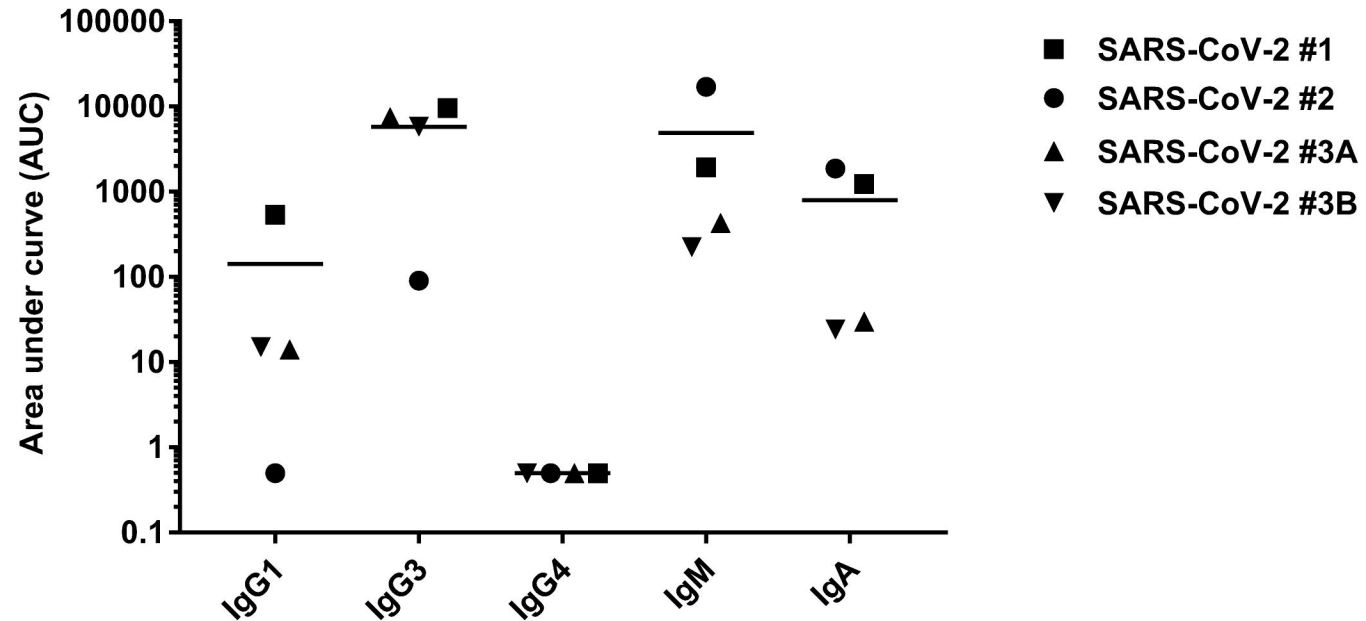
- ■ SARS-CoV-2 #1
- ● SARS-CoV-2 #2
- ▲ SARS-CoV-2 #3A
- ▼ SARS-CoV-2 #3B

PROTOCOL TITLE:

COVID-19 Sero-Eepidemiologicyal Study: Immune Response to COVID-19 Exposure and Vaccination.

Tracking and Characterizing the Immune Response to COVIDovid-19 Exposure and Vaccination

Pilot Study: Does Immune Response to SARS-CoV-2 Inform Covid-19 Virulence?

PRINCIPAL INVESTIGATOR:

Paul Scheet, PhD
Department of Epidemiology
713-745-2470
PAScheet@mdanderson.org

VERSION NUMBER/DATE:

*Modification*Version 3; *January 18*November 9, 20210.

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1	08/22/209/11/20	Addition of collaborator, Brianain Chang, PLM Inclusion of CLIA serology test; Z-code for blood collection	
2	11/1109/20	Minor logistics modification to collect data for EPIC registration submitted on 09/11/20. Resulted in re-review of whole protocol based on concerns Throughout, based on reviewers concerns regarding enrollment of MD Anderson employees. Extensive revisions made to address all concerns.	Yes
3	01/18/21	Throughout. Addition of co-Chairs and collaborators, per Dr. Litton guidance Merging of COVID-19 aspects of 2020-1054, (PI Wargo; merging of employee aspects of 2020-0533, PI Chaftari). Wordsmithing of Modifications of objectives for clarity and to cover 2020-1054 and collection of data for2020-0533	Yes

Revised: August 7, 2019

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		Inclusion of extracellular vesicle analysis, collaborator Kalluri Change of logistics; blood collection volumes; Merging of baseline questionnaire(s) Update of flyer, consent; added Appendix Update of consent C and D	

- This protocol was presented to the CCLT (Covid-19COVID-19 Core Leadership Team) onf May 8th, 2020.
- It received approval for submission to the IRB.
- Modifications to this activated protocol include , including merging with other studies protocols that intended to enroll employee participants, per have been made following a collaborator's meeting with the IRB (Dr. Jennifer Litton and Mark Chambers) on January. 13th, X, 2021

PROTOCOL TITLE:

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~~COVID-19~~ Sero-~~E~~epidemiologic~~y~~al Study: Immune Response to COVID-19 Exposure and Vaccination.

~~Tracking and Characterizing the Immune Response to COVIDovid-19 Exposure and Vaccination~~

~~Pilot Study: Does Immune Response to SARS-CoV-2 Inform Covid-19 Virulence?~~

1.0   Study Summary

Study Title	Sero-~~E~~epidemiologic~~y~~al Study: ~~Tracking and Characterizing the~~ Immune Response ~~-to~~ ~~Covid-19~~COVID-19 Exposure and Vaccination~~.~~
	~~Pilot Study: Does Immune Response to SARS-CoV-2 Inform Covid-19 Virulence?~~
Study Design	Prospective ~~non-interventional~~, longitudinal study enrolling an employee-based convenience sample with collection of biospecimens and survey data~~to collect epidemiology, serology and biomarker data~~ for study of ~~associated with Covid-19~~COVID-19.~~sero-epidemiologic Covid-19 study utilizing an employee-based convenience sample~~
Primary Objective	Track and ~~c~~Characterize humoral and cellular ~~the immunity~~e ~~response~~ to ~~COVIDovid-19~~ ~~through use of Support testing of in-house and commercially-developed immune assays~~ to optimize longitudinal evaluation of ~~immune~~ response to SARS-CoV-2 ~~ity to Covid-19~~ exposure and vaccination
Secondary Objective(s)	• Collect longitudinal~~Prospective~~ data on participant symptomology, pre~~ior~~ and/or post ~~regardless of~~ ~~in those without or following~~ vaccination
	• ~~Study~~ Study ~~status~~
	• ~~Study individual~~ individual characteristics (molecular, genetic, health history etc.) that underly variable symptomology and immunity to COVID-19~~ovid-19, before and after vaccination~~
	~~—~~Correlate fecal microbiome diversity with dietary patterns, serology and cellular immunity~~and other immune biomarkers with gut microbiome diversity and dietary patterns~~
	•
	• ~~Cumulative incidence, titer and persistence of Ab~~
	• ~~Percent with Ab but no prior symptomatology~~
	• ~~Fraction that display future seroprotection~~
	• ~~Individual characteristics that underly variable symptomology and immunity to Covid-19~~SARS-CoV-2
	• Power of in-house developed serologic assays compared to other/commercial assays for diagnosis, prognosis and management~~versus other available Covid-19 serologic assays~~

Revised: August 7, 2019

PROTOCOL TITLE:

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Research Intervention(s)	N/A
Study Population	MD Anderson Employees working on site and within the clinical moat
Sample Size	71250500 (51000250 prospective participants and 250 archived biospecimens)
Study Duration for individual participants	2- 3 years for prospective component, depending on serology, cellular immunity , immune biomarkers results and vaccination status
Study Specific Abbreviations/ Definitions	• Ab: Antibody • CCSG: Cancer Center Support Grant • AIM: Assessment, Intervention, Monitoring • PGC: Population Genomics Core • PRIME-TR: Program for Innovative Microbiome and Translational Research • IMT: Immunotherapy Platform • FU: Follow-up • BRTC: Behavioral Research Treatment Center • CLIA: Clinical Laboratory Improvement Amendments

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2.0   Objectives*

2.1   Goal

Collect prospective survey data and ~~blood~~ biospecimens to support optimization of in-house developed humoral and cellular immune assays for longitudinal evaluation of immune response to ~~Covid-19~~COVID-19 exposure and vaccination~~assays for evaluation of immune response to SARS-CoV-2~~ in a pilot enrolling ~~study of "healthy"~~ MD Anderson employees.

2.2   ~~O~~Objectives

- Collect longitudinal data on participant symptomology, pre and/or post vaccination
- Study individual characteristics (molecular, genetic, health history etc.) that underly variable symptomology and immunity to COVID-19
- ~~*Study individual characteristics that underly variable symptomology and immunity to COVID-19*~~
- Correlate fecal microbiome diversity with dietary patterns, serology and cellular immunity
- ~~*2.2*~~ Power of in-house developed serologic assays compared to other/commercial assays for diagnosis, prognosis and management

1) ~~Conduct baseline and follow-up surveys to assess healthy participants' prior and subsequent Covid-19 symptomology~~
2) ~~Employ serological assays to determine cumulative incidence and titer of SARS-CoV-2 Ab over study period~~
3) ~~Determine fraction with positive serology but no prior symptomatology~~
4) ~~Correlate Ab persistence and titer with reinfection, seroprotection and virulence~~
5) ~~Study individual characteristics that may modify response e.g., epidemiologic variables, chronic medical conditions, genetic background, gut microbial diversity, and dietary habits~~
6) ~~Compare power of in-house developed serologic assays versus other available Covid-19 serologic assays~~

2.3   Hypothesis

In-house developed immune assays for ~~Covid-19~~COVID-19 will outperform currently available serology ~~antibody~~ tests and provide more comprehensive information on extent of ~~immune~~ response and potential for future immunity.~~.~~

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3.0 Background*

3.1 The world is in the midst of a ~~Covid-19~~COVID-19 pandemic.[1] As of 0~~1~~5/12~~1~~/21~~0~~, the Johns Hopkins COVID-19 dashboard shows 91,383,544~~4,148,034~~ confirmed cases and 1,956,752 ~~284,536~~ deaths worldwide.[2,3] The US is currently leading these statistics, with ~~1,339,819~~ 22,784,091 cases and 379,551~~79,894~~ deaths.

Estimates for the transmissibility of SARS-CoV-2 range from Ro (reproduction rate) = 1.4-4.0.[4] The fatality rate is reported to vary by age, demographics, underlying health conditions, and even pollution; yet, this statistic can be difficult to quantitate as the denominator relies on the reach of ~~Covid-19~~COVID-19 testing.

The exponential increase in global cases and clear evidence of community transmission has challenged world leaders to navigate unchartered territory – how to balance maintenance of favorable economies while protecting the health of the population?

In attempts to curb the spread of ~~Covid-19~~COVID-19, countries including the US have implemented varying degrees of social distancing. Taking cues from the scientific community, state governors and other county and city officials are commanding individuals don masks in public. Stop-at-home measures have been variably mandated for the public at large including all employees deemed "non-essential." The risk benefit ratios of reopening economies and reactivating the workforce needs to be carefully considered.

~~Covid-19~~COVID-19 is a novel coronavirus family member and as such vaccine development has only just begun.[5] In the interim, the medical community must rapidly adapt and evolve best approaches to care for those critically ill patients needing intensive care. In parallel, the scientific community races to develop and repurpose therapies, and characterize infectivity, susceptibility, and the potential for host immunity.

It is well established that following viral infection, the host mounts an immune response. Indeed, based on this general knowledge, trials are underway to treat currently symptomatic ~~Covid-19~~COVID-19 patients with convalescence plasma. Yet, research is at an early stage and we do not have evidence that such plasma can neutralize SARS-CoV-2. Moreover, many individuals are testing PCR-positive for SARS-CoV-2 in the absence of ~~Covid-19~~COVID-19 symptoms.[6] We do not know if such asymptomatic carriers have developed immunity to SARS-CoV-2, and if so, to what extent. There is also some data indicating that re-infection with SARS-CoV-2 may be possible.

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[1] PMID: 32226295
[2] *coronavirus.jhu.edu/map.html*
[3] PMID: 32087114
[4] https://www worldometers info/coronavirus/#repro
[5] PMID:32238757
[6] https://www nih gov/news-events/news-releases/nih-begins-study-quantify-undetected-cases-coronavirus-infection

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To address gaps in knowledge regarding immunity, scientific teams and commercial endeavors are actively developing serology tests for SARS CoV-2. Yet, to date, such efforts have not yielded reliable products. As leaders in immunology research, MD Anderson has risen to the challenge and a number of initiatives are well underway with new assays ~~to~~ develop~~ed~~ to better characterize ~~ELISA-based assays to quantify~~ host immune response to SARS-CoV-2. ~~Such assays may focus on the SARS-CoV-2 multi-functional surface spike protein that is essential for viral entry in addition to other pathobiology.~~

The current epidemiology study is designed to support and leverage emerging serology assays. For rapid validation of SARS-CoV-2 serology tests and to advance our understanding of ~~Covid-19~~COVID-19 immunity, we will draw on convenience cohorts. The first cohort will be comprised of MD Anderson Employees. Such an approach has many benefits, including readily available email addresses and the potential for aggregated data to inform our institutions operational strategy.

Host genotype impacts risk of viral infection and clinical presentation.[7,8] Host factors involved in coronavirus family entry and replication have previously been uncovered.[9] More recently, the ACE2 receptor has been characterized as the receptor binding protein for SARS-CoV-2 and genetic variants of ACE2 may underlie interindividual susceptibility.[10][11] In addition, new evidence presents a role for host integrins during SARS-CoV-2 entry.[12] Genotype can impact viral entry, intercellular viral transmission, immune response and clearance amongst other factors. Thus, study of host genotype promises to shed light on molecular mechanisms that underlie differing susceptibility to ~~Covid-19~~COVID-19 infection, pathology, severity of symptoms, clinical outcomes and immunity among varying populations. Accordingly, we plan to conduct genotyping studies with the intent of contributing to consortia efforts.

In August 2020, the Division of Pathology Laboratory Medicine (PLM) started to offer ~~Covid-19~~COVID-19 serology testing on the Abbott Architect platform. This commercial assay tests for ~~IgG~~ antibodies against the SARS-CoV-2 nucleocapsid (N) protein and can provide information on past exp~~osu~~o~~e~~re.[13] Although highly immunogenic, detection of host antibodies to the N-protein will not provide any information on future immunity to ~~Covid-19~~COVID-19. We will use the Abbott Architect assay as a comparator in this study.

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[7] PMID:32322956
[8] PMID:31781155
[9] PMID:28643204
[10] PMID:32249956
[11] PMID:32305506
[12] PMID: 32130973
[13] PMID:32381641

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There is a growing appreciation of the role of the gut microbiome in health and disease. Indeed, microbes within the gut can shape overall immunity[14] and response to vaccination.[15] Furthermore, factors that impact the microbiome, such as diet and antibiotics, can have downstream effects on immunity. A component of this study will identify gut microbiome signatures and dietary patterns associated with enhanced immunity following vaccination. either before or after Covid-19 vaccination.

Extracellular vesicles (EVs) are shed by all cells and are contained in all biological fluids.[16] They provide molecular insight in various diseases, and their cargo inform on physiological and pathology changes.[17] EVs are also shed by bacteria (BEVs) and are found in the blood stream.[18] To inform on the immunological and microbiome profile changes associated with Covid-19COVID-19 infection and vaccination, another component of this study will profile EVs and BEVs in the plasma and feces of the study participant, pre- and post-vaccination.

3.2   No preliminary data has been collected for the epidemiologic components of this study. However-, several novel newly developed serologic assays that will be applied to this study have already been developed by collaborators under approved Drs. Kalluri, Wargo and Yee with approval under IBC protocols:

RM00004441-RN00: Evaluating the seroprevalence of SARS-CoV-2 antibodies in an asymptomatic or subclinical population at MD Anderson Cancer Center, PI, Jennifer Wargo.

RM00004423-RN00: Development of neutralization antibody test for SARS-CoV-2. PI, Cassian Yee.

RM00000518RMXXX: PI, Raghu Kalluri

3.3   The current study will help address critical gaps in our understanding of SARS-CoV-2 virulence and pathobiology. The data will help explore whether so called "Immunity Passports" for Covid-19COVID-19 can be issued for certain segments of the population. With the recent FDA Of concern, vaccine development against other coronavirus family members have not been forthcoming and there are indications that re-infection with SARS-CoV-2 may be possible. Accordingly, such research is paramount to help inform imminent and future health, business and economic decisions.Emergency Use Authorization of Pfizer and Moderna Covid-19COVID-19 vaccines, we now have an earlier opportunity to track and characterize immunity before and after vaccination.

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[14] PMID: 27383982
[15] PMID: 31491384
[16] PMID: 32029601; PMID: 27035812
[17] PMID: 32307267 PMID: 31776460
[18] PMID: 33060855; PMID: 32142907

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4.0 Study Endpoints*

4.1 N/A

5.0 Study Intervention

5.1 N/A. There is no study intervention. This protocol does not provide any treatment and is not designed to evaluate and implement changes in behavior.

6.0 Procedures Involved*

6.1 This ~~is a~~ prospective epidemiologic study ~~that~~ will implement online questionnaires and collect ~~blood~~ biospecimens. Analysis of blood samples will include ~~for subsequent analysis of~~ host immune response to SARS CoV-2 infection and/or vaccination, ~~and g~~ Genome wide scan~~s~~ of germline DNA and other molecular analyses. ~~will also be conducted.~~ Stool biospecimens will be analyzed for microbiome signatures via 16S rRNA gene sequencing.

6.2 Research Outline (Fig. 1)~~)~~
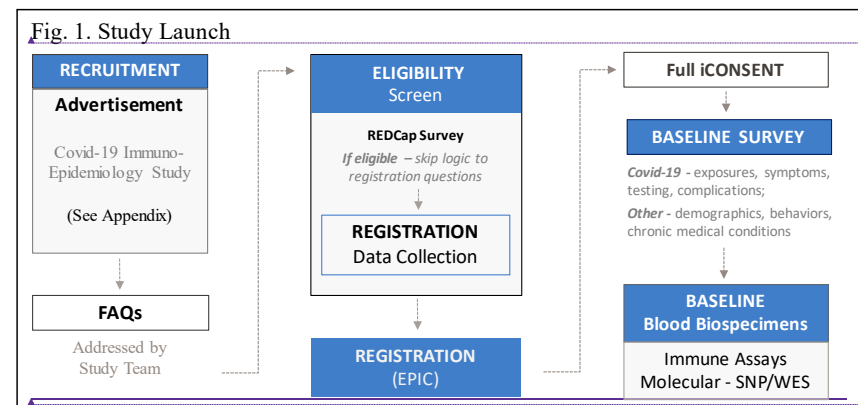
Fig. 1. Study Launch

The following subsections will be detailed in Section 6.2: , Procedures:

- o Survey ~~M~~methodology, 6.2.1
- o Eligibility Screen and Registration to EPIC , 6.2.2
- o Baseline Questionnaire, 6.2.3
- o Study Follow-up and Timelines, 6.2.4~~3~~
- o CLIA Serology Assay, 6.2.5
- o B~~iospecimen~~lood ~~C~~collection, 6.2.6
- o I~~Serology / i~~mmune Assay and Profiling, ~~monitoring~~6.2.7
- o Genome-wide Survey of Genetic Variation, 6.2.8~~scans~~

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- o Stool MmMicrobiome Profiling, 6.2.9
- o Extracellular Vesicle Analysis (stool and bloodand molecular analysis), 6.2.10
- o

6.2.1 Survey Methodology

Questionnaires will be developed in house and implemented in electronic format.

SSurvey questions will be coded in Research Electronic Data Capture (REDCap) and study data collected and managed using the applications electronic data capture tools hosted at MD Anderson.[19, 20]

Surveys will be staged throughout the project. Based on participant response, we may develop more in-depth follow-up instruments in key areas to obtain additional information. Survey instruments will may evolve as the research community-at-large gleans more information about Covid-19COVID-19 susceptibility and outcomes. All questionnaires will be submitted for IRB-approval before implementation.

We will draw on validated instruments to identify measures relevant to Covid-19COVID-19. Such tools may include ongoing in-house epidemiologic studies (see section 25) as well as those posted to reputable sites e.g., CDC; NIH Covid-19COVID-19 relevant research instruments. We may also generate new questions.

6.2.2 Eligibility Screen and Registration to EPIC

SURVEY 1 (*Appendix A*): A REDCap survey link (*Appendix A*) will be emailed to interested individuals to prescreen for study eligibility (Fig. 1). Eligibility criteria are detailed in Section 10. If a potential participant qualifies, skip logic will link the survey to key questions needed for the registration team to determine if a record for the subject already exists in in EPIC. Registration in EPIC will allow the team to use iConsent for the study.

For individuals who do not meet the eligibility criteria, built-in skip logic will ensure that the survey comes to a closeends and no further information is will be collected.

Registration into EPIC will follow all standard MD Anderson processes. It will be carried out by qualified and trained staff in the Behavioral Research Treatment Center. Following registration, participants will be consented to the study before any study-related data and biospecimens are collected and study consent (Fig 1). , two additional questionnaires will be sentd to participants:

6.2.3 Baseline Questionnaire

SURVEY 2 (*Appendix B*): The baseline questionnaire (*Appendix B*): is will be developed in REDCap and has two main components. Participants will be sent a second survey to

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~~collect baseline information related to the study. Th~~e first component ~~is survey will~~ collect~~s~~ data on demographics, chronic medical conditions, selected medications, key lifestyle factors~~behaviors~~, prior ~~Covid-19~~COVID-19 symptoms, diagnosis, testing, vaccination etc. This section ~~component~~ should take about 5 minutes to complete. The second component asks ~~It links to~~ several questions relevant to gut health followed by the

~~SURVEY 3 *(Appendix C):* A link to the web-based ~~NCI Dietary Screener Questionnaire (DSQ) and will require about 15 minutes of time. If not completed, the link will be resent once each week for a total of 3 reminders ~~follow-ups.times. which is preceded by several~~ ~~questions micriobiome on use of will also be emailed sent to participants.~~

6.2.4   Study Follow-u~~pp (FU)~~ and Time lines,

Fig 2. Study follow-up

PILOT FOLLOW-UP SCHEDULE: Survey and Biospecimens
- Seroconversion, persistence and reinfection response monitoring

BASELINE SEROLOGY → STUDY END

q 2 months ± 1 week
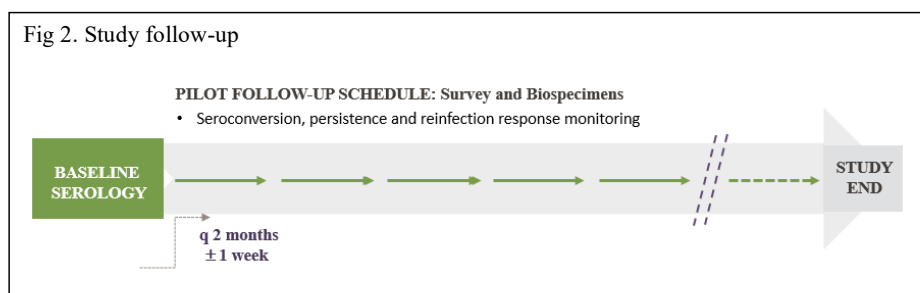
SURVEY 3+ FOLLOW-UP

~~(Follow Up).~~ Follow-up surveys are under development and will be approved prior to distribution. They will be tailored to monitor future exposure to SARS-CoV-2, ~~and/or~~ development of ~~Covid-19~~COVID-19 symptomology, changes in vaccination status, adverse events following vaccination, and other relevant factors. If participants select 'No" to new events (e.g. exposures, symptoms, diagnosis, positive PCR test), skip logic will abbreviate the survey. Those selecting "Yes" to any of the aforementioned events will be presented with more questions to collect additional details.

~~Participants will receive a follow-up survey each 2 months ± 1 week. Participants may~~ ~~remain on study for different periods of time.~~ The goal is to monitor for any changes in immune response, symptomology and adverse events over time in participants regardless of vaccination history. We will ~~and to~~ determine how ~~if~~ the presence of circulating antibodies, ~~or~~ other immunity (e.g. T-cells), and microbiome signatures impact ~~may~~ ~~prevent~~ future symptomology.

Timeline~~s~~

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Year 1 : follow-upIntended FU times are indicated in **Table 1.** However, the study needs to have built in flexibility due to the rapidly changing situation. Subjects will join the study with differing histories of Covid-19COVID-19 exposures, symptoms and Covid-19 vaccination; history /of and Covid-19 susceptibility to Covid-19COVID-19 will change during the course of the study. Also, emerging SARS-CoV-2 variants may decrease the effectiveness of the currently available vaccines and we intend to monitor this situation. The consent document will note that at Possible changes in follow-up timing/frequency may occur., if necessary, to complete the goals of the study. , will be included in the consent.

- The brief monthly follow-up survey for unvaccinated subjects (Table 1) participants will query changes in monitor changes in vaccination status. If subjects indicate that they do not intend to get vaccinated, this question will be rolled into the q4 month follow-ups. *If consented to sharing vaccination recordsdata (Employee Health), step this step may be unnecessary.*

- For subjects starting study with only 1 vaccination shot, monthly FU (as above) may be necessary if there is any delay If there is any delay at MD Anderson in receiving the second dose. vaccination shot, it may be necessary to follow up each month, as above.

Table 1

Covid-19 Vaccination Status	FU (Month)	REDCap QX	Blood Collection
from baseline			
Unvaccinated	q 1	√	na
	q 4	√	√
post 2nd dose			

Table 1. Year 1 Follow-up

COVID-19 Status	Follow-up (Month)*	REDCap QX	Blood Collection
from baseline			
Unvaccinated	q 1	√	na
	q 4	√	√
post 2nd dose			
Vaccination complete	1	√	√
	5	√	√
	11	√	√
* initial follow-up contact will occur +/- 1 week of schedule listed			

Covid-19 Vaccination Status	FU Month	REDCap QX	Blood Collection
(from baseline)			

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Year 2 follow-up will be each 4 months for unvaccinated participants and each 6 months for those with complete vaccination. Timelines for follow-up need to remain flexible to due to accommodate for the rapidly changing situation. Subjects EAs each participant will join the study with differing histories of ent past exposures, or symptoms and Covid-19 vaccination; and ; this history / and future Covid-19 susceptibility will change during the course of the study. Possible changes in follow-up timing/frequency will be included in the consent. Moreover, emerging SARS-CoV-2 variants may decrease the effectiveness of the currently available vaccines and we intend to monitor this situation E Thus, each participant will have , and have different future susceptibilities and this can dictate to future exposures and symptoms, this will requiringe different lengths of follow-up.

Unvaccinated	q 1	√	na
	q 4	√	√

Table 1

Covid-19 Vaccination Status	FU (Month)	REDCap QX	Blood Collection
	(from baeline)	√	√
Unvaccinated	q 1	√	na
	q 4	√	√
	(post 2nd dose)		
Vaccination complete	1	√	√
	56	√	√
	112	√	√

The brief monthly survey for unvaccinated participants will check for changes in vaccination status. If participant consents to sharing of Covid-19 vaccination data from Employee Health, this step may not be necessary.

For those with a single vaccination shot at the time of study, and if If there is any delay at MD Anderson in receiving the second vaccination shot, it may be necessary

Unvaccinated:

Each month: request *vaccination status update (*survey via REDCap). *Notes: ,* if participant consents to sharing of Covid-19 vaccination data from Employee Health, this short survey maywill not be necessary

- Each 34 months: Covid-19 testing /and symptom update (via REDCap): surveyblood collection. *Note, if participant consents to sharing of vaccination data from Employee Health, this short survey will not be necessary*

If unvaccinated: Each 3 months

Post Following Second Dose of Vaccination completed (ed (2 doses received): ion:

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Pilot Study: Does Immune Response to SARS-CoV-2 Inform Covid-19 Virulence?

2 doses):

  1 1 *month* (survey via REDCap); blood collection: 0,2,

  636 *months, 5 months, 1212 months* post initial vaccination

Note, if there is any delay n receiving second vaccine doses at MD Anderson, 1 dose received:

Each month: vaccination status update (survey via REDCap). *Note, if participant consents to sharing of Covid-19 vaccination data from Employee Health, this short survey may not be necessary*

Moreover, emerging SARS-CoV-2 variants may decrease the effectiveness of the currently available vaccines and we intend to monitor this situation.

While participants may remain on study for different periods of time, weWe anticipate most will be followed for about 2 years.

Time Lines

Following Second Dose of Vaccination:XXXX

: 0,2,6,12.

At any of the follow-ups, should a participant be in quarantine and/or temporarily suspended from on-site work, biospecimen collection will be placed on hold until the employee meets current institutional guidelines for and returns to on-site work. In such cases, the participant may will receive more frequent follow-up by surveys each month to determine changes in Covid-19COVID-19 status and clearance for on-site work.

Note – all Covid-19COVID-19-related institutional guidelines and on-site entrance surveys that assess an employee's clearance for work e.g. following travel, will over-ride the biospecimen schedule.

6.2.2 5   *CLIA Sserology Aaassay*:

The Abbott Architect Covid-19COVID-19 Antibody test will be ordered through EPIC and linked to the research blood Z-code orders (*Section 6.2.3*) so that only a single visit to the diagnostics labs will be required for the baseline and each follow-up timepoint. The blood drawn for CLIA testing (1 lavender tube) will remain within Pathology Laboratory Medicine (PLM) for assay of Covid-19COVID-19 serology using PLM SOPs. The CLIA test will be performed at baseline and at any other timepoints deemed necessary as the data emerges.

6.2.63 *Biospecimen Collection*

- Blood cCollection by Z-code

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Venipuncture for *Sections 6.2.4* will be performed by well-trained phlebotomists in MD Anderson's diagnostic labs using the Z-code mechanism. This will ensure all current MD Anderson SOPs and PPE guidelines are followed.

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Research b~~AFor research assays, up to 5 tubes (about 4 tablespoons) of b~~l~~Two teaspoons of b~~lood will be drawn from ~~biospecimen~~ each ~~per~~ participant at baseline and each follow-up. ~~This will cover the CLIA serology testing (section 6.2.52) and research blood.~~

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The following vacutainers will be used: ~~will be collected in~~ lavender top (1~~1~~ tube), red top (1 tube) and ~~or~~ green top (2~~3~~ tubes), light blue top (1 tube). ~~vacutainers.~~ Biospecimens will be transported by courier and delivered to the appropriate drop point for pickup by designated lab staff associated with Epidemiology's Population Genomics Core (PGC), an MD Anderson approved iLAB~~s~~. ~~I,~~If / when the clinical moat is removed, designated PGC staff can ~~maywill~~ pick-up samples directly.

On receipt of biospecimens, a record for each sample will be created in BIMS for tracking purposes by PGC staff on the Delegation of Authority log (*Section 7*). One tube of blood will be retained ~~with the~~ in the PGC for the epidemiologic lab studies. Other ~~All other~~ blood biospecimens will be transferred ~~sent~~ to collaborators directly, or via the ~~the~~ Immunotherapy Platform (IMT) for initial processing and then ~~and~~ distributed~~ution~~ to collaborators, for completion of ~~performing the~~ serology and immune assays.

Any b~~BThe b~~iospecimen~~s~~ processing by PGC personnel will occur ~~will be processed~~ in the Biosafety level 2 (BSL-2) lab as covered under ~~by staff PGC personnel with appropriate training, divided into aliquots~~ as appropriate ~~and labeled with the study ID that excludes all PHI. BiospecimensAliquots will be entered in the institutional BIMS for tracking.~~

~~IRB 2020-0584 is now covered under~~ approved IBC protocol RM00004731-RN00. The IBC has not requested heat inactivation of the biospecimens collected herein. However, if this changes in the future, blood biospecimens will be subjected to heat inactivation at 56ºC for 30 mins in a heating block prior to processing.

Dr. Paul Scheet will serve as the overall steward of the biospecimens. The PIs and collaborators ~~of the immunology labs~~ (Wargo, Ka~~l~~hluri, Yee, IMT) will become the stewards of any ~~processed~~ biospecimen sample that is transferred to their lab for processing or immune testing. All transferred biospecimens will be tracked in BIMS.

- *Stool Sample Collection*

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Directly following consent, participants will be handed or mailed a fecal collection kit, complete with instructions, together ~~along~~ with a stamped, ~~addressed~~ return envelope addressed to ~~to return to laboratory of~~ the Program for Innovative Microbiome and Translational Research (PRIME-TR) laboratory, located on the 5th floor of the South Campus Research Building 4. ~~Instructions for at home retrieval of stool ....~~The PRIME-TR team will conduct all f~~F~~ollow-up to obtain return of stool samples (*Appendix C*, email; Appendix D, call ~~and DX~~). Follow-up will be once per~~each emails will be~~

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sentdix , as necessary, each week week for a maximum of 7 times. Frequency.the mailed kit will be

collected using an at home retrieval kit.

6.2.74 - *Immune Assays and Profiling*

Blood biospecimens will be evaluatedassayed for the host's immune response to key SARS-CoV-2, be it after symptomatic or asymptomatic infection and/or before and after vaccination. surface proteins (IBC protocols RM00004441 RN00 and RM00004423 RN00, *Section 3.2*). Immune assays willmay. encompass the SARS-CoV-2 including but not limited to recombinant full--length spike and protein and/or nucleocapsid proteins, and spike receptor-binding domain etc,. Immune aand nucleocapsid. Such aAssay formats will include but are not limited to s include quantitative ELISA, flow cytometry, mass cytometry (CyTOF), scRNASeq, ELISelispot -and or other such immunoassays.X, Y, Z amongst other formats.

Collaborators labs-will perform those novel serology and immune assays s already set up and standardized in their own laboratories (see IBC protocols, section 3.2). labs. Other immune monitoring assays available at MD Anderson will be performed by the Immunotherapy Platform, per PA13-0291.

- Sensitivity/Specificity

  Biospecimens collected herein will be used to further optimize sensitivity/specificity analysis of in-house developed assays. The results from the research immune assays will be compared to those derived from:

  a. Abbot Covid-19COVID-19 antibody Architect platform (Section 6.2.2);

  b. Participant's response to the baseline survey questions regarding results of any Covid-19COVID-19 nasal swab tests performedperformed, and other data collected therein (*Section 6.2.1*, Survey 2 and follow-up surveys);

  c. Results from Covid-19COVID-19 nasal swab tests and vaccinations processed by Employee Health, provided -if participant agrees to share data during consent to the study.

- Neutralizing Potential

  Dr. Yee's lab (*Section 3.2*) has developed in vitro neutralization assays to determine the potential for participant plasma to block SARS-CoV-2 binding/entry into host cells. Assays employ ACE2 expressing / transformed cells coupled with either recombinant SARS-CoV-2 proteins, ege.g. spike, or pseudo-typed lentivirus vectors expressing luciferase/green fluorescent protein and SARS-CoV-2 spike protein or its receptor binding domain.

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Dr. Kalluri's lab also has a pseudovirus neutralization assay that employs vesicular stomatitis virus (VSV) expressing surface SARS-CoV-2 spike protein.

Other neutralizing assay formats for this assay may be developed and applied to the biospecimens collected over the course of the in this study. for ine future analysis.

- Immune Monitoring (Fig 2)

After completion of baseline serology, longitudinal blood biospecimens will be collected for: 1) evaluation of Ab titer and persistence; 2) monitoring of seroconversion and seroprotection; 3) documentation of new exposures / symptoms, diagnosis; 4) determination of a future immune response; 5) genetic mosaicism over study time-frame.

Our goal is to We will attempt to schedule and collect baseline bBlood biospecimens will be collected within preferably within 72 hours, but no more than 1 week of consenting the subject to the study ,the following baseline or follow up survey completion. Follow-up schedules for immune testing will be identical to those described in Section 6.2.1.

6.2.853. *Genome-wide Survey of Genetic Variation*

Germline genomic DNA will be isolated in the PGC or other facility using standard kits e.g. Qiagen. kits. Quality and quantity will be assessed by Nanodrop and PicoGreen. As SARS-CoV-2 RNA has been detected in blood, BSL procedures will be implemented.[21]

- Genotyping

Scanning for known common genetic variants (minor allele frequency, MAF ≥ 1% to 5%) using high-throughput DNA microarrays (e.g. Illumina BeadChip) will allow characterization of single nucleotide polymorphisms (SNPs), indels, and copy number variants (CNVs), and inference of detectable clonal chromosomal mosaicism. The study maywill utilize both off-the shelf Infinium products with preselected SNPs as well as microarrays that afford custom design to close any gaps in coverage of specific genomic areas relevant to Covid-19COVID-19. Arrays of interest used may will consider coverage of exomes, ancestry markers, chronic disease e.g. diabetes, immune system, clinical variants, amongst others.

Targeted genotyping and validation will be performed using other technologies e.g. TaqmanTaqMan. All genotyping will be conducted in-house by the Population Genomics Core (PGC, *Section 25*).

- Genome Sequencing

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[21] PMID: 32159775

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Whole genome sequencing (WGS), whole exome sequencing (WES), and targeted deep, next-generation sequencing will enable: 1) identification of rarer variants (MAF <1.0%), including in transcribed regions of the genome; 2) smaller CNVs than can be detected with DNA microarrays; and 3) inference of acquired point mutations (e.g. clonal hematopoiesis). These studies will complement the genomic scans performed on the Illumina bead chips.

The PGC staff have extensive experience in preparing exome libraries for next generation sequencing. Completed libraries will be submitted to the Advanced Technology and Genomics Core (ATGC) at MD Anderson for conduct of WGS/WES on HiSeq instrumentation (Section 25) or outsourced.[22] No PHI will be shared with the ~~ATGC~~ sequencing lab~~s~~.

6.2.6 ~~-~~Stool M~~M~~icrobiome Profiling~~Analysis~~

Fecal samples collected will be subjected to a standardized protocol for 16S rRNA gene sequencing with data processing and analysis through PRIME-TR, with banking of residual sample for future use (for metagenomic sequencing and other studies).

6.2.7 Extracellular Vesicle Analysis (stool and blood)

To inform on the immunological and microbiome profile changes associated with vaccination, we will also profile EVs and BEVs in the plasma and feces of the study participant, pre- and post-vaccination. Profiling will include evaluation of proteomic and nucleic acids (miRNA, mRNA), and in the case of BEVs, 16S sequencing.

~~Directly following consent, p~~participants will be mailed provided with a fecal collection kit (either at the time of blood draw or via mail) with a stamped, addressed envelope to return to laboratory of the Program for Innovative Microbiome and Translational Research (PRIME-TR) located oin the 5th floor of the South Campus Research Building 4.~~

6.3 This is a relatively low risk study. Approaches to minimize loss of confidentiality are described in *Section 17*. Phlebotomy-associated risks will be minimized by employ of well-trained and experienced staff in the diagnostic labs. All data collection instruments will be reviewed by the IRB before implementation. Questions have been identified for the baseline survey (*Appendix B*). Follow-up surveys are under development.

6.4 The following types of data will be collected:

- **Survey responses:**

  Eligibility criteria (*Section 10 and Appendix A*)

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[22] https://mdanderson ilabsolutions com/service_center/show_external/3628

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Registration data*: (Appendix A).* ~~We have worked with two EPIC registration teams to identify the minimal PHI needed for registration of employee participants in EPIC: 1) Team registering employees for asymptomatic Covid-19 testing by nasal swab; 2)~~The registration team ~~Team~~ in the Behavioral Research Treatment Center (BRTC) ~~who have registered employees in the Tobacco Treatment Center. The BRTC~~ will ~~be~~ register~~ing~~ the employee participants into EPIC for 2020-0584. Ahead of registration, we will collect limited PHI to support the registration team in this process.

Baseline questionnaire: myriad data, including *but not limited to*:

o   Demographics: gender, age, ethnicity, race, etc.

o   ~~Covid-19~~COVID-19 History: symptoms, ~~confirmed~~ exposure~~.~~; ~~confirmed~~ diagnosis, testing, vaccination; hospitalization; complications; therapy received; time of convalescence

o   Lifestyle Factors: e.g. smoking history - never, current, former; alcohol use; dietary habits (food frequency), etc.

o   Medical History: chronic illness – respiratory, cardiovascular, diabetes; pneumonia; hypertension; ~~— including~~ medications; etc.

o   ZIP Code: To allow correlations with residential demographics, ~~Covid-19~~COVID-19 hotspots, environmental exposures, etc. ~~We will request ZIP Code+4.~~

o   ~~*Dietary Questionnaire: food intake frequency of various food categories*~~

• **Serology and Immune Assays:** results obtained from the immune assays described herein, including the research assays covered under the IBC protocols (*Section 3.2*) and the ~~-~~Abbot~~t~~ CLIA assay. ~~(*Section 6.2.3*).~~

• **Genomic Data:** SNP and WES data using the platforms described herein.

6.5 Long-term follow-up will involve longitudinal collection of blood ~~and stool~~ biospecimens for immune~~,~~ ~~and~~ molecular ~~and gut microbiome~~ studies; implementation of additional questionnaires to assess ~~self-reported Covid-19~~COVID-19 exposure, symptoms, diagnosis, testing, complications, vaccination etc.

7.0 Data and Specimen Banking*

7.1 This is not a banking protocol. However, upon completion of the goals described herein, restricted access to any residual biospecimens and data will be made available to other investigators for C~~ovid~~OVID-19-related research.

All blood biospecimens will be entered into and tracked within the Institutional Biospecimen Information Management System (BIMS). This platform complies with NCI's best practices for biorepositories and affords centralization of resources, security and transparency. All ~~Each~~ biospecimen~~s~~ will be ~~assigned,~~ labeled *with a unique Study*

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ID devoid of PHI prior to storage and any distribution. *and stored in freezers within the PGC under a unique Study ID devoid of PHI.*

Only designated PGC lab staff listed on the Delegation of Authority will have access to the stored biospecimens. Collaborator labs will request biospecimens from the PI and PGC staff will distribute samples devoid of PHI.

7.2   The survey-related data will be stored separately from the biospecimens. All survey data will be stored in the REDCap database, which is hosted on a secure server by MD Anderson Cancer Center's Department of Oncology Care & Research Information Systems. This application is accessed through Secure Socket Layer (SSL). Only those with rights to login to the REDCap instrument will have access to this data.

7.3   The Department of Epidemiology has established Standard Operating Processes (SOP) for access to biospecimens and data collected from existing IRB approved protocols that consent said materials to future research. The same SOP will be followed for any remaining samples that are consented to future research in protocol 2020-0584.

Requests for biospecimens/data are made via REDCap. An initial feasibility form is completed for the database manager to check if samples and or data is available. If available, the requesting PI will complete a formal request through REDCap; this includes upload of: project description, IRB protocol, Waiver of Informed Consent. The Data and Biospecimen Access Committee convene to review the research and ensure the protocol covers the proposed science and lists the protocol, in this case 2020-0584, as a data/biospecimen source. Only de-identified data are provided unless the protocols are approved otherwise.

8.0   Sharing of Results with Subjects*

At this time, there are no plans to release serology data/results to participants, even if such data was generated under non-CLIA conditions. The protocol does include Covid-19COVID-19 serology testing in a CLIA environment. Thus, it might be reasonable to release CLIA results in the future as subjects would benefit from this data, a measure of prior Covid-19COVID-19 exposure. However, the CLIA test only measures antibodies against the SARS-CoV-2 nucleocapsid protein, whereas vaccinated individuals with no prior SARS-CoV2 exposure would only have antibodies against the viral spike protein, per vaccine design. Such a scenario would yield a negative CLIA test and generate much confusion for the participant. -

The sequencing conducted in this protocol is not performed under CLIA conditions. However, if any incidental finds with potential clinical significance are found, we will consult with the IRB, legal and other key institutional initiatives engaged in such research (e.g. efforts led by Genomic Medicine) to follow all current guidance, policies and practice regarding if, when and how to communicate such data.

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~~The Abbott Covid-19 serology test performed "in house" by PLM will be conducted under CLIA conditions. This allows for sharing of results and we will employ MyChart/Epic to release this data.~~

~~Microbiome results – are they performed under CLIA, and if not can they be released?~~Participants will receive a report by mail on the composition of their fecal microbiome within 180 days of enrollment (with metrics describing the diversity and composition of bacteria within their ~~gut~~fecal microbiome).

9.0   Study Timelines*

9.1   Individual subject participation will begin immediately following their consent to the study. ~~For subjects with negative serology at baseline, and negative serology at each subsequent follow-up, they will remain on study for 24 ± 1 month. For subjects with baseline seropositivity, and positive serology at each subsequent follow-up, they will remain on study for 24 ± 1 months. M~~For all others, maximum individual participation may extend up to 3 years ± 2 months. The detailed follow-up timeline is described in *Section 6.2.4.*

9.2   The study is expected to evolve over a duration of up to 3 years with the precise duration depending on: speed of recruitment and number and timing of those who have changing serology status; how the ~~Covid-19~~COVID-19 pandemic evolves over time; vaccination status and efficacy. ~~In the best-case scenario, we will have a vaccine within the next 18 months. In the interim, w~~We expect to be recruiting currently healthy participants with the following ~~Covid-19~~COVID-19 related histories: 1) no prior exposure, 2) prior exposure with little to no symptoms; 3) prior exposure with symptomology of varying severity. Each of these participants may or may not have been ~~; and 4) ultimately,~~ vaccinated against ~~Covid-19~~COVID-19. ~~if such efforts are successful.~~

10.0   Subject Population*

10.1   This pilot study includes a prospective cohort (N-~~500~~~~100~~~~250~~) as well as access to archived biospecimens (N-250). The population will include adults of all ethnicities and any race.

The prospective component will utilize convenience sampling to develop a non-probability cohort of MD Anderson employees. ~~for immune testing and survey administration.~~This will provide for cost savings and allow for more rapid launch of the protocol in this time-sensitive environment.

Archived biospecimens (N=250) will be accessed for the sole purpose of testing the sensitivity and specificity of the immune assays. All archived biospecimens must have collection dates prior to the ~~Covid-19~~COVID-19 outbreak and have been consented for future research. There is no intent to access any other data associated with the archived biospecimens.

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10.2   Specific Eligibility and Ineligibility Criteria

<u>Prospective Cohort</u>

Eligible
- MD Anderson employees
- Must be working on site at the main campus and within the clinical moat.
- Must be able to donate blood without leaving the clinical moat
- Age 18 and older

Ineligible
- Students, trainees, fellows
- Any collaborator, study team member, or staff delegated to work on the project

<u>Retrospective Component</u> *Section 17.1.*

Eligible
- Archived biospecimens from epidemiologic studies
- Age 18 or older
- Informed consent already provided for banking of biospecimens for future research
- Biospecimen archived on dates prior to outbreak of ~~Covid-19~~<u>COVID-19</u>

10.3   This protocol will NOT seek to consent the following vulnerable populations: adults unable to consent; individuals who are not yet adults (infants, children, teenagers); ~~pregnant women;~~ prisoners. <u>The study eligibility screen does not screen out p</u>~~P~~<u>regnant women and they will not be excluded from the study.</u>

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11.0 Vulnerable Populations*

11.1   This study WILL seek to consent a cohort of MD Anderson employees; such subjects need to be protected from potential for coercion or undue influence related to participation, and susceptibility to unforeseen consequences emanating from participation. To address these issues, the Study PI will email all department chairs prior to starting study recruitment. They will be reminded that all decisions and actions related to employment, including but not limited to performance evaluations, career advancement , assignments, and PTO approvals must NOT be impacted (favorably or unfavorably) based on an employee's study participation decision or status.

To further minimize the possibility for coercion, all participation decisions (to participate, not participate, withdraw from study) made by employees will not be disclosed to, and will be protected from disclosure to, anyone other than members of the research team. Such information will not be used by the study team for any non-study purpose. The following study-related SOPs will help guard against disclosure:

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- A dedicated email address (EpiCovidImmunity@mdanderson.org) for all study-related participant communications has been created. The only team members with access to this email include the database manager, research interviewers and scientific admin lead for the study.

- Interested participants will be mailed a REDCap link to complete eligibility and registration. Only the database manager, research interviewers, and scientific admin lead will have viewing access (REDCap security features detailed in 17.2). The database administrator is the only one who can export data. Registration data will be exported into an excel file and uploaded to a secure password-protected folder. The registration team will access this folder to input data into EPIC. The registration team, by definition will have access to PHI.

- All communications regarding stool collection, mail out of kits and follow-up for return of kits, will occur through a dedicated email (Primetr@mdanderson.org), ... managed by staff with the appropriate human subjects protection training within PRIME-TR. tr.the Program for Innovative Microbiome and Translational Research.

The database administrator, research interviewers, registration team and scientific admin lead have all completed the appropriate Human Subjects Training. All are aware of the utmost need to keep participant identity and all communications confidential.

12.0 Local Number of Subjects

12.1   The pilot study will launch with recruitment of up to 500 participants for the prospective component of the study

We will also identify 250 blood biospecimens that were previously collected and banked for future research prior to the start of the Covid-19COVID-19 pandemic. These biospecimens will come from collections banked in Epidemiology and will serve as controls for the research serology assays.

13.0 Recruitment Methods

13.1   Recruitment will take place at MD Anderson, including online and in the form of posters and flyer handouts (see *Section 13.4*). Flyers will be handed out at appropriate locations; this includes the CPB2 site reserved for testing asymptomatic employees for Covid-19COVID-19 virus. Posters will be displayed onsite at MD Anderson approved locations. Online recruitment will include posting to the internal Website (e.g. Employee notes), and inclusion in slide announcements at institutional meetings.

Timeframe for recruitment: as soon as feasible following IRB approval of the protocol.

13.2   The current version of the protocol targets only MD Anderson employees that self-identify as currently working on-site and within the clinical moat.

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13.3   Potential subjects will be identified from amongst MD Anderson employees using the strategies outlined in *Section 13.1*.

13.4   A recruitment Ad has been generated and uploaded in ePRTCL. This Ad will be distributed using any of the methods / formats outlined in *Section 13.1*. All advertisements and modes of distribution will be approved by communications before recruitment begins.

13.5   There will be no monetary incentives associated with this study.

14.0   Withdrawal of Subjects*

14.1   We do not anticipate any circumstance under which subjects will be withdrawn from the research without their consent.

14.2   We will put procedures in place should any participant request withdrawal from the protocol. Actions will depend upon the extent to which the participant wishes to withdraw:

- Complete Withdrawal:
  - o Requesting exclusion of all biospecimens and data in study analyses; AND
  - o No further contact, including collection of longitudinal data through REDCap survey instruments

- Partial Withdrawal:
  - o Exclusion of biospecimens OR data from analysis; OR
  - o Allowing retention of collected data and biospecimens but requesting no further follow-up

Blood Biospecimen Actions: The laboratory manager in charge of the BIMS collection will identify all samples and derivatives thereof, pull from storage and destroy based on best practices. They will identify any samples that have already been distributed to study collaborators and communicate the requirement to the appropriate faculty and personnel. Best efforts will be made to destroy distributed samples. If samples have already been included in assays, it will not be possible to destroy them.

Stool Samples:

Data Actions: The staff analyst/manager charged with database administration, tracking and QA/QC, will identify all relevant data records. This may include survey, serology, PCR or genomic datasets and analyses thereof, depending on the extent of withdrawal. Each collaborator, and if applicable their delegate staff member, will be informed of the participants request and the procedures that need to be taken. Every accommodation will be made to flag such data for permanent deletion.

Whenever possible and reasonable, if data has already been included in any unpublished analyses, it will be extracted; analyses will be rerun with omission of such dataset(s). It

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~~COVID-19~~ Sero-~~E~~epidemiologic~~yal~~al Study: Immune Response to COVID-19 Exposure and Vaccination. ~~Tracking and Characterizing the Immune Response to COVIDovid-19 Exposure and Vaccination Pilot Study: Does Immune Response to SARS-CoV-2 Inform Covid-19 Virulence?~~

will not be possible to remove data from published analyses. However, all data will only be reported in aggregate. This will prevent identification of results at the individual level.

15.0 Risks to Subjects*

15.1 There are no foreseeable risks associated with completing the online questionnaires although some subjects may find certain personal or medical history questions to be sensitive. There are foreseeable risks associated with phlebotomy, including pain, bleeding, bruising, fainting, and possibly infection at the site of venipuncture.

15.2 There are no unforeseeable risks to subjects consenting to and participating in the study.

15.3 There are no procedures that would yield risks to an embryo or fetus should any subjects become pregnant in the future.

15.4 This protocol may be associated with risks to laboratory staff involved with biospecimen collection and subsequent molecular / immune profiling. ~~serology testing.~~ However, all such personnel will receive the appropriate training and operate under MD Anderson's evolving guidelines and SOPs.

16.0 Potential Benefits to Subjects*

16.1 Participants taking part in this study have the opportunity for increased altruism and may experience long-term personal benefits (health and psychology) associated with contributing to efforts targeted at halting the pandemic to promote global public health.

16.2 This study is not interventional and therefore there will be no possibility for benefits from an intervention. ~~The However, the~~ protocol ~~does include~~ has been updated to allow for ~~additional Covid-19 serology testing in a CLIA environment. Thus, and thus it might be reasonable to . For participants being tested in this manner, it will be possible to release CLIA results in the future as . Under these circumstances, subjects would benefit from this data, such data as it is a measure of prior Covid-19 exposure. However, the CLIA test only measures antibodies against the SARS-CoV-2 nucleocapsid protein, whereas vaccinated individuals with no prior SARS-CoV2 exposure would only have antibodies against the viral spike protein, per vaccine design. Such a scenario would yield a negative CLIA test and generate much confusion for the participant. Therefore, at this time we do not intend to release the CLIA test results to the participant.~~

17.0 Data Management* and Confidentiality

17.1 All ~~Serology and immune profiling~~ data ~~generated for assay optimization~~ will be generated in conjunction with the IBC protocols described in Section 3.2.

Sensitivity and specificity analysis of the serology assays will rely on incorporation of historical biospecimens collected prior to the ~~Covid-19~~COVID-19 outbreak; such samples will be collected under IRB approved epidemiology protocols LAB98-040, CPN91-001, LAB02-174. Receiver-Operator curves (ROC) will be generated to evaluate Area Under the Curve (AUC) with 95% confidence interval (CI) and test performance.

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We will track and monitor seropositivity trends and other immune profiling and ~~to~~ perform all manner of correlative analysis against: survey data responses including exposures. ~~;~~ symptoms. ~~; and where possible Covid-19~~COVID-19 history, testing and vaccination, ~~; status as confirmed by PCR;~~ behaviors. ~~;~~ chronic disease, dietary factors; and ~~gut~~fecal microbiome signatures, ~~e.t.c~~etc.~~.~~

As a primary objective of this pilot study, ~~w~~We will estimate the proportion of SARS-CoV-2 Ab positivity at the baseline and seroconversion rate during follow-up. These proportions will be estimated together with 95% Binomial-based exact CIs. With ~~25~~00 subjects, we can estimate the Ab positivity rate (p) at the baseline with an exact 95% CI with half width = 0.03 for p = 5%, 0.04 for p = 10%, and 0.047 for p = 15%. Thus, if estimated prevalence p = 10%, the 95% CI will extend from 6.6% to 14.4%.

~~O~~Secondary objectives are to uncover underlying factors and interactions that may explain: variability in incidence, ~~:~~

- ~~P~~presence, absence, and titer, and ~~of SARS-CoV-2 Ab~~

- ~~l~~persistence ~~Longevity~~ of circulating Ab against SARS-CoV-2~~; Ab;~~

- ~~A~~ability of positive serology and other immunity (e.g. T-cells) ~~anti-SARS-CoV-2 Ab~~ to confer ~~Covid-19~~COVID-19 immunity, and to what extent. ~~(i.e. subsequent exposure yields mild of more severe symptomology)~~

We will also perform independent correlative analysis between genome scan and survey data to uncover genetic variables that may predict risk, symptomology, and immune response to ~~Covid-19~~COVID-19. Due to the limited sample size, this will likely entail merging of deidentified data with other larger national datasets collected for similar purposes. We will seek IRB approval prior to such data sharing which will be completed under fully executed MTAs.

Summary statistics including measures (central tendencies, variation, etc.) will be generated and reported for all survey questions using standard statistical tools (SAS, SPSS, STATA, Excel and/or R). Logistic regression and proportional hazard models will be employed to determine associations between survey measures and longitudinal serology data, ~~and~~ genetic variation, immune and molecular profiling.

~~Gut~~Fecal microbiome profiles will be assessed for diversity, composition, structure, and functional characteristics. Within-sample (alpha) diversity will be computed using the inverse-Simpson index, and across-sample (beta) diversity will be computed using weighted Unifrac distance. Differences in alpha diversity across groups (e.g., subjects achieving seroprotection or seroconversion vs. those that do not) will be visualized using side-by-side boxplots and tested using a Mann-Whitney test, and differences in beta diversity across groups will be visualized using a principal coordinates analysis (PCoA) plot and tested using permutational analysis of variance (PERMANOVA). To identify

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specific microbiome features that differ across groups, we will apply linear discriminant analysis effect size (LEfSe).[23] (PMID 21702898). Functional characteristics of the microbial community will be obtained from 16S profiling using PICRUSt or from WGS profiling, if available, using HUMAnN2.[24] (PMID: 23975157, 30377376).

Dietary intake will be summarized by intake of micronutrients, macronutrients, and total energy intake (kcal). These measures will be correlated with microbiome diversity and specific microbiome features using Spearman rank correlation.

To associate covariates with seroconversion or seroprotection status, for each vaccine antigen, we will fit univariate and multivariate logistic regression models. In multivariate models, we will adjust for age and baseline antibody level, as these factors have been previously associated with vaccine response.[25] (PMID: 21668661). To identify a microbiome signature of seroconversion, we will first filter the microbiome features to those with sufficient prevalence. We will then fit a lasso logistic regression model with centered-log-ratio transformed (CLR) microbiome abundances as covariates subject to selection, age and baseline antibody levels as fixed covariates, and seroconversion status as the response variable. We will also consider including dietary covariates if these are found to be significantly associated to seroconversion in univariate logistic regression. Model performance will be evaluated using measures of predictive accuracy (sensitivity, specificity, AUC) obtained from cross-validation. Blood will also be banked and used for additional immune profiling studies and assessment of response to additional vaccines.

17.2 **Survey Data:** All survey data will be collected in REDCap. REDCap is a secure, web-based application with controlled access designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless downloads to common statistical packages; and 4) procedures for importing data from external sources.

REDCap has undergone an annual Governance Risk & Compliance Assessment (since May 2014) by MD Anderson's Information Security Office and found to be compliant with HIPAA, Texas Administrative Codes 202-203, University of Texas Policy 165, federal regulations outlined in 21CFR Part 11, and UTMDACC Institutional Policy #ADM0335.

Those having access to the REDCap databases for this study include the PI, REDCap database administrator, and only critical research team personnel who need access to complete the goals of the study. Users are authenticated against MDACC's Active Directory system. The application is accessed through Secure Socket Layer (SSL). If the need arises, REDCap support staff will be consulted for help with administration.

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[23] PMID: 21702898
[24] PMIS: 23975157 PMID: 30377376
[25] PMID: 21668661

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The administrator of the study database has privileges for Project Design and Setup; ability to assign user rights; create Data Access Groups; export the full dataset.

The PI and statistician only have access to export de-identified data fields, which includes exclusion of all free form text, date/time fields and identifier fields. All other study team members have no access to export data. The registration information will be exported and shared with ~~uploaded to a password-protected OneDrive folder for use by~~ the EPIC registration team through their dedicated email address: *BRTC-staff@mdanderson.org*. In cases where a stool kit needs to be mailed, key staff associated with the PRIME-TR, ~~Program for Innovative Microbiome and Translational Research,~~ and who are listed on the Delegation of Authority log, will be provided participant mailing addresses. ~~The~~.

All protected health information (PHI) will be removed from the study-related baseline, dietary questionnaire and follow-up survey data when ~~it is~~ exported from REDCap for analysis. Any deidentified study data outside of REDCap will be stored in password-protected databases and maintained on MD Anderson servers behind the firewall.~~.~~

Following publication study data will be archived in REDCap. Since study data may be useful for future research studies performed under separate IRB approved protocols, study data will be archived indefinitely in REDCap. Since REDCap is a secure electronic database with controlled access, and because participant identifiers may be needed to link study data to data from other sources under future IRB approved protocols, participant identifying information will be retained in the archived database.

EPIC Data: Employee records will be locked in EPIC using the "Brake the Glass" security feature. Anyone trying to access locked records needs to provide their MD Anderson credentials and a valid reason for needing access. Clinical data in EPIC is not openly available to MD Anderson the Employer. To gain access, the Employer would need to acquire a signed "Release of Information (RoI)." In contrast to other operations, this study will not be collecting an RoI on behalf of MD Anderson the Employer.

Lab Data: All blood biospecimens will be tracked in the institutional BIMS (Sections, 6.2.3; 7.1). ~~Microbiome~~Fecal samples will be cataloged and stored by PRIME-TR within the Wargo lab~~....~~. All lab data records will be labeled and tracked using unique identifiers that are devoid of PHI. Only the PI, ~~and~~ database ~~/study~~ managers and other key personnel who need such access to complete the goals of the study will have access to the key that links the unique identifiers with PHI. All data generated will be housed within databases behind MD Anderson's firewall and on password-protected institutional drives / folders. ~~and OneDrive folders.~~ Use of approved Cloud-based solutions will enable secure sharing of data and facilitate project management.

Study Team: A Delegation of Authority log will be generated to outline the roles of all study team members involved in the project. Each collaborator that leads a component of this protocol will take responsibility for ensuring staff members are adequately informed on delegated roles, research procedures, duties and functions.

Revised: August 7, 2019

PROTOCOL TITLE:

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Each member of the research team - PI, collaborators, delegated laboratory and analytic staff – who have or are granted access to PHI will first complete all institutionally-approved and mandated Human Subjects Protection Training.

17.3   Regarding quality control (Q/C), all survey data collected in REDCap will be associated with audit trails. The inherent Data Quality module will allow for routine checks and analysis of data discrepancies e.g. missing fields, throughout the project.

Q/C of serology and other immune and molecular profiling ~~related~~ data will be performed by the collaborators and under the IBC protocols described herein (*Section 3.2*).

All ~~molecular~~ genetic profiling data will be generated in the PGC Core under current SOPs and Q/C procedures.

17.4   The protocol PI will provide oversight for management and distribution of blood ~~deidentified~~ biospecimens and associated survey data. <mark>All protocol collaborators and their study teams will only have access to de-identified biospecimens for conduct of immune assays.</mark> Laboratory managers reporting to the PI will be responsible for initial receipt of biospecimens and subsequent ~~the~~ distribution ~~and receipt~~ of deidentified samples to collaborators and the IMT platform.~~.~~ ~~biospecimens.~~

- Technical staff will log receipt of blood biospecimens in the Epidemiology lab using the institutional BIMS, including filling out tracking to document the precise storage location. Use of biospecimens in experimental studies will be tracked through BIMS.

- Biospecimens will be stored in the freezer farms (-30_oC, -80_oC) as appropriate at the main campus research labs (e.g. PGC Core, serology/immunology-testing labs) involved with this research. ~~Following biospecimen collection, all samples will be entered into BIMS.~~ Locations of ~~unprocessed and processed~~ biospecimens will be tracked in BIMS. ~~as they are distributed to the research labs.~~ Cryogene Lab may be used for long-term storage.

~~Only delegated staff and database managers directly involved in the study and needing access will be granted viewing or editing rights to these files, as appropriate.~~

Any residual biospecimens will be banked for use in future C~~ovid~~OVID-19-related research. Should any biospecimens remain after the study has concluded, a DBAC will ~~be assembled to~~ review and prioritize requests for use of any remaining banked resources.

- The current study is highly topical and multiple groups on both national and international levels are dedicated to determining immunity to ~~Covid-19~~COVID-19 in convalescence. We anticipate that the genetic data generated herein will be combined in subsequent meta-analysis to increase power. In such cases, all data will be stripped of all identifiers according to HIPPA regulations.

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- We plan to upload deidentified genomic scan data to the NIH database for Genotypes and Phenotypes, dbGaP. Individual level data will be made available only under controlled access and to PIs with IRB approved protocols for general research use. Use of this data will require collaboration with the primary study investigator.

18.0   Provisions to Monitor the Data to Ensure the Safety of Subjects*

This is a minimal risk study and safety data will not be collected.

19.0   Provisions to Protect the Privacy Interests of Subjects

19.1   As part of the consent process, participants will be informed that their data and any remaining biospecimen will be banked for future research related to the immune response to Covid-19COVID-19. It will also be communicated that they can withdraw from the study at any time.

19.2   Participants will be informed that all survey questions posed are directly relevant to the study at hand. Should they feel uncomfortable with answering any of the questions in the baseline and follow-up surveys, then they may move to the next question and leave it unanswered. All questions are mandatory in the eligibility and registration questionnaires.

Phlebotomy is a standard procedure and all blood draws will be performed by experienced staff operating under MD Anderson SOPs. Thus, the subject should be placed at ease and any physical comfort will be minimized.

19.3   The PI will be responsible for granting study team member access to identifiable information. Such access will only be approved if needed to complete the goals of the study.

20.0   Compensation for Research-Related Injury

20.1   N/A; this is a minimal risk research project that will obviate the need to consider research-related injury.

21.0   Economic Burden to Subjects

21.1   As participants will be employees who are currently working on-site, there will be no additional costs related to transportation /parking for blood draw visits. They are not expected to incur any other type of economic burden.

22.0   Consent Process

22.1   Informed consent will be required for this study.

For any electronic survey conducted prior to full consent (eligibility/registration survey), the "Authorization for Participation in Research" statement will proceed the actual questionnaire. In the event that subjects proceed move to the "next page." following the Authorization, this will be documented as an individual's consent to participate in the study activities that precede full consent.

Revised: August 7, 2019

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For study procedures involving biospecimen collection, we will follow the SOP: Informed Consent Process for Research (HRP-090).

As ~~an~~ optional procedure~~s~~, participants will be asked if they would be willing to share any current and future results from ~~Covid-19~~COVID-19 testing and vaccination performed at MD Anderson Employee Health. Gathering such data would enhance the interpretation of the serology and immune profiling assays performed in the laboratory. ~~under development.~~

Non-English Speaking Subjects – N/A

At this time, we will not recruit non-English speaking subjects.

23.0 Process to Document Consent in Writing

23.1 We will be following "SOP: Written Documentation of Consent (HRP-091). Unless there is opportunity for in-person consent, the consent process will be conducted remotely according to any of MD Anderson's most current and evolving practices and ~~Covid-19~~COVID-19 guidance ~~using e.g.~~ institutional iPhones and KLIC-SIGN, ~~or~~ Zoom ~~e.t.e.~~etc. ~~according to MD Anderson's current practices and Covid-19 guidance.~~

23.2 *N/A* – not waiving the requirement to obtain written documentation of consent.

23.3 The consent document has been uploaded in the consent space in ePRTCL.

24.0 Setting

24.1 The study will be conducted at MD Anderson main campus. Access to all buildings and areas will be guided by the evolving CCLT/ELT restrictions and approval.

25.0 Resources Available

25.1 For the prospective component of this study, we will draw on a convenience sample of up to 50~~250~~0 MD Anderson employees working on-site and within the clinical moat. We should not have a problem in reaching this goal. For the retrospective component, we will draw on archived biospecimens from long-standing epidemiologic case-control studies that have been consented for future research and banked by the PGC.

Questionnaire Development and Implementation

- Epidemiology has substantial expertise in developing, implementing and managing comprehensive questionnaires for past and ongoing prospective cohorts and case-control studies, including the former Patient History Database.

- For question selection, we will draw on ~~review~~ existing Epidemiology measures and readily available national/international validated collection instruments (dietary

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screening questionnaire), including but not limited to the Covid-19COVID-19 research tools at the NIH Office of Behavioral and Social Sciences Research.[26]

- We have previously worked closely with the AIM CCSG core on REDCap and Qualtrics platform surveys. We will draw on their expertise as necessary to drive the project forward in a timely manner.

Serology and Immune Profiling Assays

The MD Anderson faculty body has vast expertise in immunology and immunotherapy development that spans over different divisions and departments. The current protocol taps into some of this expertise and is designed to support key Covid-19COVID-19 serology / immune profiling initiatives being conducted within the cancer center. Thus, there are ample resources available for optimization of the studies described herein. novel Covid-19 serology and immune profiling assays.

Genotyping, WES

- The PI and Epidemiology faculty have vast collective knowledge in identification of common and rare variants associated with chronic diseases and phenotypic traits.

- Genotyping and library preparation for WES will be conducted by experienced laboratory staff associated with the PGC, an MD Anderson iLABs solution housed within Epidemiology.[27]

The WES will be performed using Agilent technology and HiSeq or comparable instrumentation within the CCSG ATGC Core.[28]

GutFecal *Microbiome* Analysis

MD Anderson's microbiome initiative is led by the Program for Innovative Microbiome and Translational Research under the direction of Dr. Wargo (collaborator). PRIME-TR has developed ample resources to support the collection and processing samples as well as data generation, processing, and analysis for microbiome applications. These resources will be made available for this study.

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[26] *https://www.nlm.nih.gov/dr2/COVID-19_BSSR_Research_Tools.pdf*
[27] https://mdanderson.ilabsolutions.com/landing/1492
[28] https://mdanderson.ilabsolutions.com/service_center/show_external/3628)

EVALUATING THE SEROPREVALENCE OF SARS-COV-2 ANTIBODIES IN AN ASYMPTOMATIC POPULATION AT MD ANDERSON CANCER CENTER

Lead and co-lead: Nadim Ajami and Eleonora Dondossola

MDACC Collaborators: Giannicola Genovese, Jennifer Wargo, Pam Sharma,

External Collaborators: Tony Piedra (BCM), Maria Elena Bottazzi (BCM), Florian Krammer (Mount Sinai), Michael Laposata (UTMB)

May 1st, 2020

(UNIVERSAL) REAGENTS

Antigens and Antibodies:

- **Receptor Binding Domain of SARS-CoV-2 (NR-52306)**
  - Plasmid grown and RBD purified in-house
  - Antigen ordered from BEI Resources (ordered, limited quantity)
  - Antigen sourced from BCM (Maria Elena Bottazzi, MTA wip)

- **Spike protein of SARS-CoV-2 (NR-52306)**
  - Plasmid grown and RBD purified in-house (ramping-up)
  - Antigen ordered from BEI Resources (ordered, limited quantity)

- **Goat Anti-Human IgG HRP (Sigma)**
- **Goat Anti-Human IgM HRP (Sigma)**
- **Rabbit SARS-CoV-2 S1 mAb (Sino Biological)**

ASSAY SETUP

PLASMID EXPRESSION, PROTEIN PURIFICATION, ASSAY SETUP

RBD-SARS-CoV-2
- BEI Resources
- Maria Elena Bottazzi (BCM)
- Eleonora Dondossola (MDACC)
- Gianni Genovese (MDACC)

POSITIVE SERA
- ITB MDACC
- Pathology, UTMB
- Tony Piedra, BCM

SPIKE-SARS-CoV-2
- BEI Resources
- Gianni Genovese (MDACC)
- Eleonora Dondossola (MDACC)

PREPANDEMIC SERA
- Jen Wargo
- Tony Piedra, BCM
- Pathology, UTMB

ASSAY UTILIZATION

SCREEN ELISA (RBD)

Day1
- Precoated and blocked plate
- Heat inactivate samples
- Predilute samples
- Sample dilution
- Secondary Ab
- Develop

Throughput: 1 operator, 760 samples/10 plates per run

CONFIRMATORY ELISA (SPIKE)

Day1
- Precoated and blocked plate
- Block
- Predilute samples
- Serial dilutions
- Secondary Ab
- Develop

1 operator, 140 samples/10 plates per run

TIMELINE & ACTIVITES

Week	Apr 27	May 4	May 11	May 18	May 25	June 1	June 8
REAGENTS SOURCING	▓	▓	▓				
ELISA SETUP		▓	▓	▓	▓		
ELISA VALIDATION					▓	▓	
TESTING							▓

LAB	ACTIVITY
JEN WARGO (MDACC)	Plasmid amplification & protein expression (RBD and spike)
ELEONORA DONDOSSOLA (MDACC)	Protein purification & ELISA Setup
GIANNI GENOVESE (MDACC)	Plasmid amplification (RBD and spike)
MARIA ELENA BOTTAZZI (BCM)	RBD
TONY PIEDRA (BCM)	Protocol details
MICHAEL LAPOSATA (UTMB)	Positive and pre-pandemic sera

COVID19 SEROPREVALENCE RESEARCH STUDY

PHASE I

ASSAY DEVELOPMENT
4 weeks

MDA – ELISA

- Quantitative
- Antigen: Receptor Binding Domain (screening), and Spike protein (confirmatory)
- Antibody: IgG and IgM
- Controls: Positive sera and mAb; pre-pandemic sera

PHASE II

ASSAY UTILIZATION
Start June 8th

SET 1

COVID19 confirmed and probable cases – samples banked at ITB

SET 2

Research, healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

COVID19 ELISA → **Positive** → Presumed Immune

COVID19 ELISA → **Negative** → Not presumed immune

IgM + ; IgG -   Resolving infection

IgM + ; IgG +   Convalescent

IgM - ; IgG +   Recovered

PHASE III

ASSAY EXPANSION
To be determined

- Expansion to wider population

- Repeat testing to determine duration of antibodies in circulation

- Development of receptor-binding inhibition assays

- Development of neutralization assays (UTMB)

2019-nCoV_N1-F2019-nCoV_N1   GACCCCAAAATCAGCGAAAT
2019-nCoV_N1-R2019-nCoV_N1   TCTGGTTACTGCCAGTTGAATCTG
2019-nCoV_N1-P2019-nCoV_N1   ACCCCGCATTACGTTTGGTGGACC
2019-nCoV_N2-F2019-nCoV_N2   TTACAAACATTGGCCGCAAA
2019-nCoV_N2-R2019-nCoV_N2   GCGCGACATTCCGAAGAA
2019-nCoV_N2-P2019-nCoV_N2   ACAATTTGCCCCCAGCGCTTCAG
2019-nCoV_N3-F2019-nCoV_N3   GGGAGCCTTGAATACACCAAAA
2019-nCoV_N3-R2019-nCoV_N3   TGTAGCACGATTGCAGCATTG
2019-nCoV_N3-P2019-nCoV_N3   AYCACATTGGCACCCGCAATCCTG

>2019-nCoV N Positive Control (sequence length = 1260)
ATGTCTGATAATG**GACCCCAAAATCAGCGAAAT**GC**ACCCCGCATTACGTTTGGTGGACC**CT**CAGATTCAACT**_GGCAGT_**AACCAGA**ATGGA
GAACGCAGTGGGGCGCGATCAAAACAACGTCGGCCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCACCGCTCTCACTCAACATGGC
AAGGAAGACCTTAAATTCCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAATTGGCTACTACCGAAGAGCT
ACCAGACGAATTCGTGGTGGTGACGGTAAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAACTGGGCCAGAAGCTGGA
CTTCCCTATGGTGCTAACAAAGACGGCATCATATGGGTTGCAACTGA**GGGAGCCTTGAATACACCAAAA**GA**TCACATTGGCACCCGCAAT
CCTG**CTAA**CAATGCTGCAATCGTGCTACA**ACTTCCTCAAGGAACAACATTGCCAAAAGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGT
CAAGCCTCTTCTCGTTCCTCATCACGTAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTCCTGCTAGAATG
GCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTGGTAAAGGCCAACAA
CAACAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGCCACTAAAGCATACAATGTA
ACACAAGCTTTCGGCAGACGTGGTCCAGAACAAACCCAAGGAAATTTTGGGGACCAGGAACTAATCAGACAAGGAACTGA**TTACAAACAT
TGGCCGCAAA**TTGC**ACAATTTGCCCCCAGCGCTTCAG**CG**TTCTTCGGAATGTCGCGC**ATTGGCATGGAAGTCACACCTTCGGGAACGTGG
TTGACCTACACAGGTGCCATCAAATTGGATGACAAAGATCCAAATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGACGCATAC
AAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAAGAAGGCTGATGAAACTCAAGCCTTACCGCAGAGACAGAAGAAACAGCAA
ACTGTGACTCTTCTTCCTGCTGCAGATTTGGATGATTTCTCCAAACAATTGCAACAATCCATGAGCAGTGCTGACTCAACTCAGGCCTAA

RP-F_RNAseP_Forward   AGATTTGGACCTGCGAGCG
RP-R_RNAseP_Reverse   GAGCGGCTGTCTCCACAAGT
RP-P_RNAseP_Probe TTCTGACCTGAAGGCTCTGCGCG

Hs_RPP30_Positive Control:
GGACTTCAGCATGGCGGTGTTTGC**AGATTTGGACCTGCGAGCG**GG**TTCTGACCTGAAGGCTCTGCGCG**G**ACTTGTGGAGACA
GCCGCTC**ACCGTGAGTTGCGGTCTCCCAGAGTCTCTGGGATGTCCCTGGAGGCTGATG
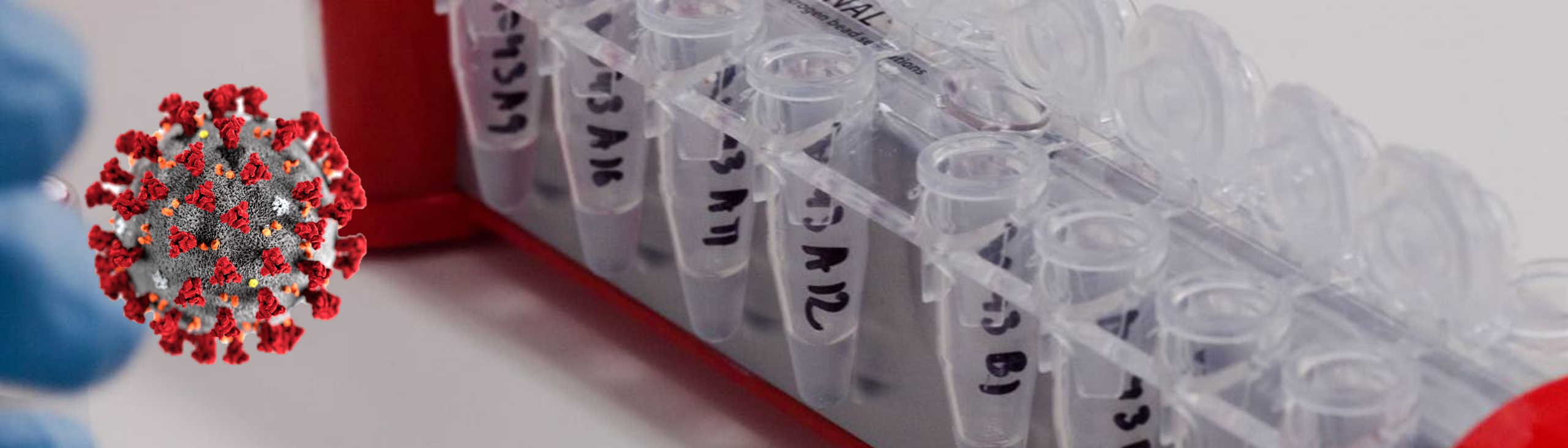
EVALUATING THE SEROPREVALENCE OF SARS-COV-2 ANTIBODIES IN AN ASYMPTOMATIC POPULATION AT MD ANDERSON CANCER CENTER

RESEARCH PROTOCOL

Leads: Eleonora Dondossola and Nadim Ajami

Internal Collaborators: Giannicola Genovese, Jennifer Wargo, Pam Sharma,

External Collaborators: Tony Piedra (BCM), Maria Elena Bottazzi (BCM), Florian Krammer (Mount Sinai), Michael Laposata (UTMB)

ASSAY SET UP
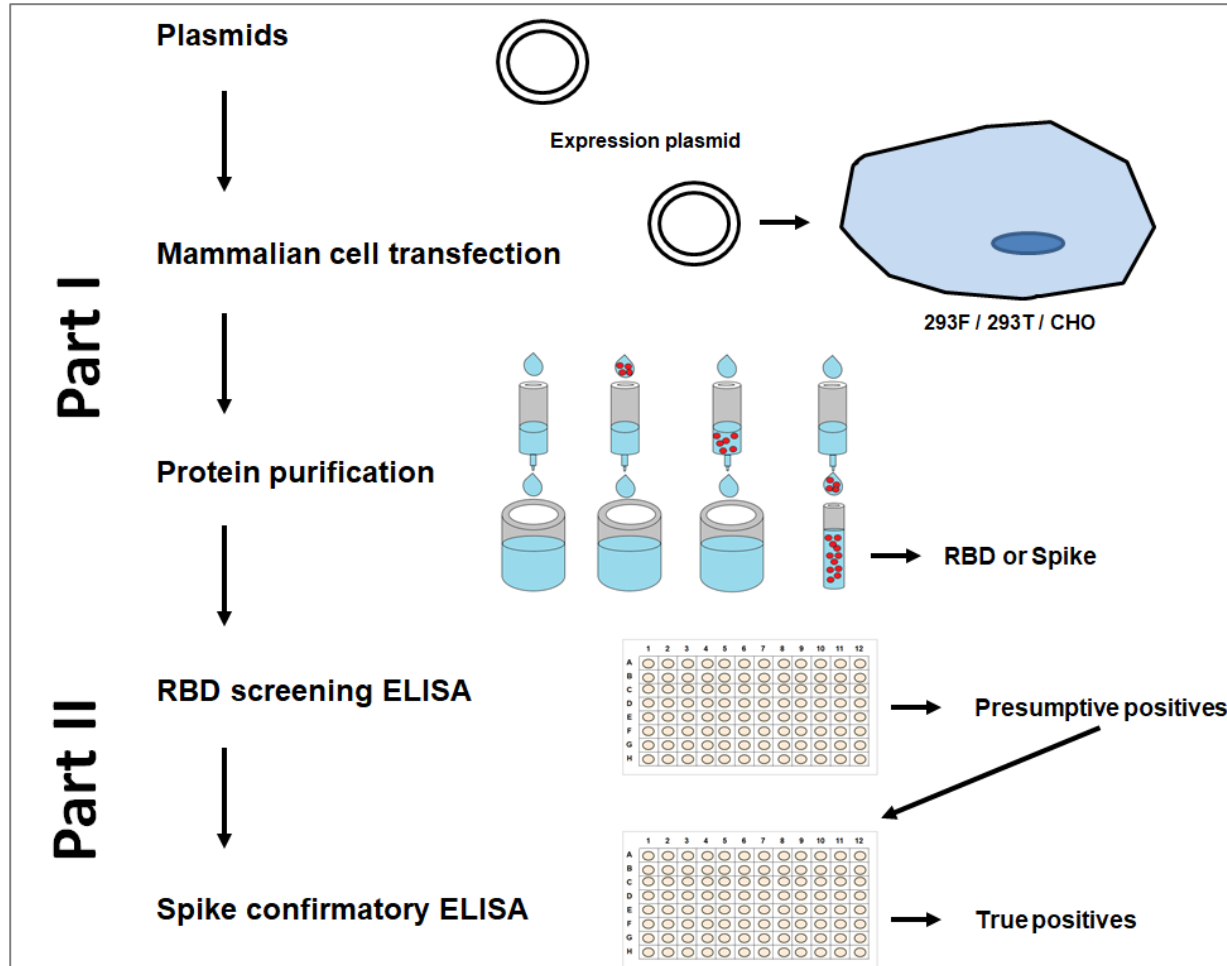
TIMELINE & ACTIVITES

Week	Apr 27	May 4	May 11	May 18	May 25	June 1	June 8
REAGENTS SOURCING	■	■	■				
ELISA SETUP		■	■	■	■		
ELISA VALIDATION					■		
TESTING						▓	▓

LAB	ACTIVITY
JEN WARGO (MDACC)	REAGENT SOURCING
ELEONORA DONDOSSOLA (MDACC)	Protein expression, purification & ELISA Setup
GIANNI GENOVESE (MDACC)	Plasmid amplification (RBD and spike)
NADIM AJAMI (MDACC)	Logistics, MTA, protocols, coordination
MARIA ELENA BOTTAZZI (BCM)	RBD
TONY PIEDRA (BCM)	Protocol details

PLASMIDS

1. Spike (Δ cleavage site)
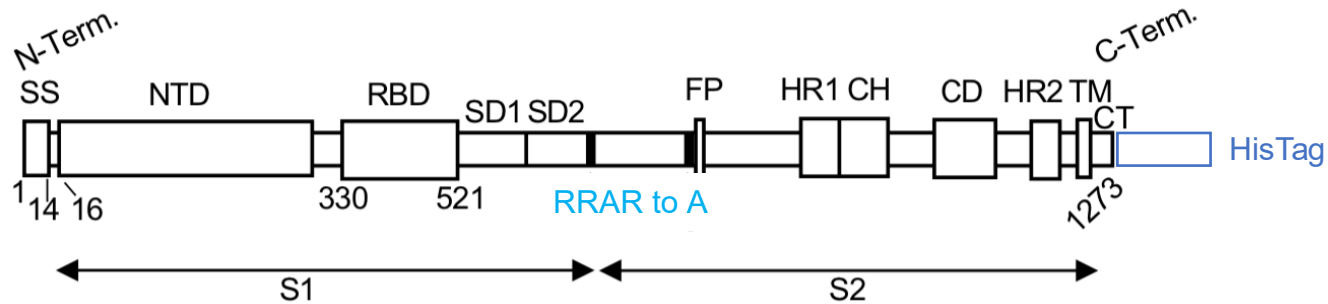
2. Spike + PP (Δ cleavage site + stabilizing Proline mutation)
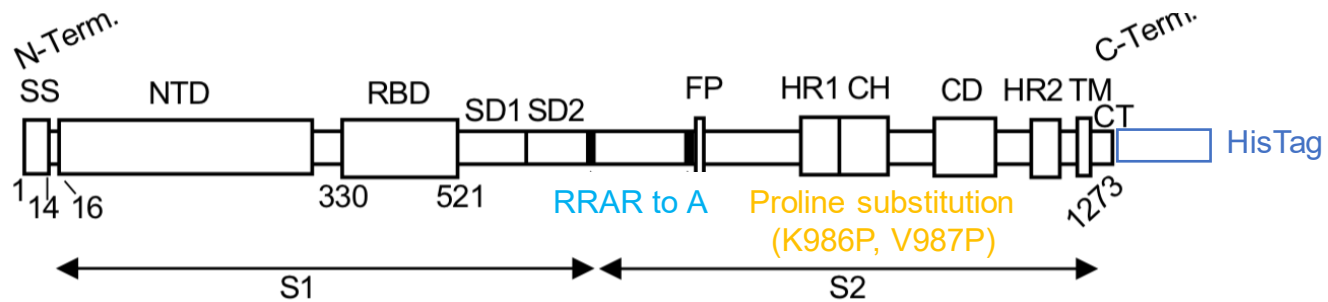
3. Receptor Binding Domain
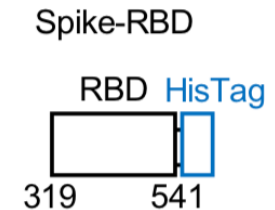
Adapted from McAndrews, Kalluri et al.

PLASMID AMPLIFICATION

Dr. Carbone (Dr. Genovese's Lab)

Spike (clone):   2460 ng/µl (1476 µg total)
Spike (pool):   1670 ng/µl (1002 µg total)

Spike + PP (clone): 1560 ng/µl (936 µg total)
Spike + PP (pool):   1780 ng/µl (1068 µg total)

RBD (clone):   2250 ng/µl (1350 µg total)
RBD (pool):   2680 ng/µl (1680 µg total)

TRANSFECTION AND PURIFICATION

Claudia Paindelli, Dr. Dondossola

Plasmid (~100 µg)
+
HEK293FT
(~250k)

TRANSFECTION   PROTEIN PRODUCTION   PROTEIN PURIFICATION
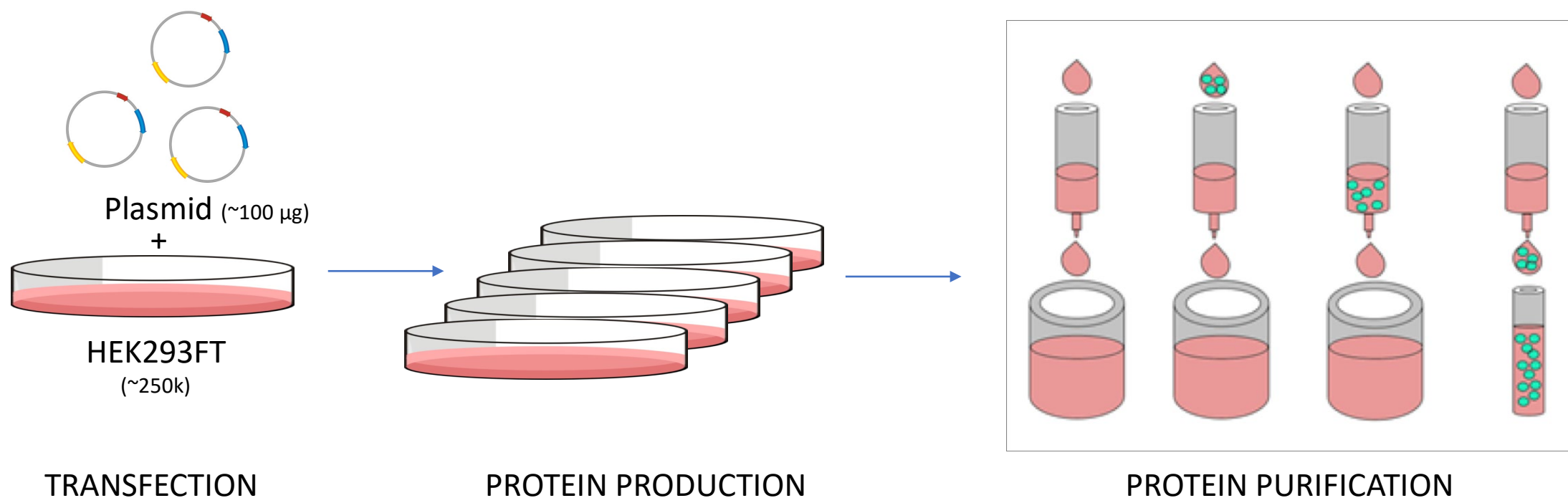
PROTEIN PURIFICATION AND CHARACTERIZATION

Claudia Paindelli, Dr. Dondossola

RBD: pilot study, 1 mg total

Spike : ongoing
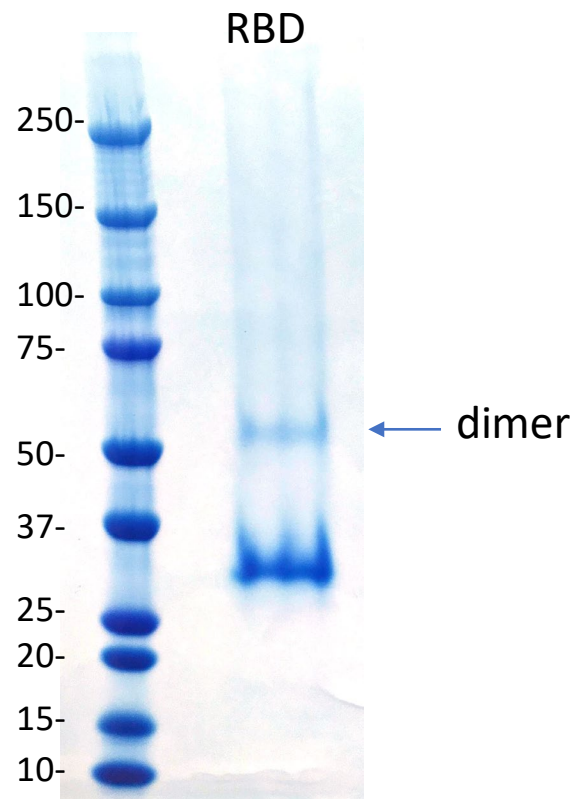
ASSAY UTILIZATION

Claudia Paindelli, Dr. Dondossola

ASSAY UTILIZATION

SCREEN ELISA (RBD)

Day1
- Precoated and blocked plate
- Heat inactivate samples
- Predilute samples
- Sample dilution
- Secondary Ab
- Develop

Throughput: 1 operator, 760 samples/10 plates per run

CONFIRMATORY ELISA (SPIKE)

Day1
- Precoated and blocked plate
- Block
- Predilute samples
- Serial dilutions
- Secondary Ab
- Develop

1 operator, 140 samples/10 plates per run

The natural history of COVID-19 in cancer patients: a comprehensive cancer center's experience

Principal investigator: Roy F Chemaly

Collaborators: Fareed Khawaja, Nadim Ajami, Jennifer Wargo, Joseph Sassine, Sam Shelburne, Pablo Okhuysen, and Ariza El Heredia

Background. Cases of novel coronavirus disease (COVID-19) were first reported in China in December 2019 [1]. SARS-COV-2 (the causative pathogen of COVID-19) has spread to the western hemisphere, and has been declared a pandemic by the WHO [2]. The clinical characteristics and outcomes associated with COVID have been described, but limited data is available on the impact of COVID-19 in cancer patients [3-7].

Objectives.

Primary objective: To describe the 60-day clinical course of cancer patients admitted for COVID-19 at MD Anderson Cancer Center.

Secondary objectives:

1) To compare clinical outcomes in COVID-19 infected Cancer patients to previously analyzed cohort of cancer patients infected with community acquired respiratory viral infections.
2) To describe changes in respiratory and gastrointestinal microbiome in cancer patients with COVID-19 and correlate changes to clinical outcomes.
3) To describe changes in respiratory virome in cancer patients diagnosed with COVID-19 correlate changes to clinical outcomes.
4) To measure RNA viral load of SARS-COV-2 in nasopharyngeal, feces and blood samples in cancer patients diagnosed with COVID-19 and correlate changes to clinical outcomes.
5) To measure changes in neutrophils count, lymphocytes count, and cytokines levels during the first 30 days of COVID-19 in cancer patients, and correlate immune response to clinical outcomes in cancer patients diagnosed with COVID-19.
6) To describe presenting symptoms and durations of symptoms in cancer patients diagnosed with COVID-19

Methods.

Study design: A prospective study to follow cancer patients who are admitted to MDA with a diagnosis of COVID-19. Enrolled patients will undergo weekly clinical follow up (over the phone, in person or via the electronic medical system) for 60 days to assess symptom progression and clinical status changes. We will also collect weekly nasopharyngeal, fecal and blood samples for 28 days (week 0, 1, 2, 3, 4), and analyze the samples as noted in table 1.

Variables of interest: We will collect patient demographics, non-cancer co morbidities, malignancy history, cancer treatment history, symptomology, radiographic findings, clinical characteristics, clinical course in the hospital and post discharge, laboratory data and antiviral therapy. We will also collect data using samples collected as shown in table 1.

Outcomes: The primary outcome of interest would be 30- and 60-day mortality rates in COVID-19 cancer patients.

Secondary outcomes include duration of symptoms, duration and severity of hypoxia, ICU admission and length of stay, hospital length of stay, severity of pneumonia, rate of mechanical ventilation, duration of mechanical ventilation support and rate of secondary bacterial infection.

Samples of interest	Nasopharyngeal samples	Fecal samples	Blood samples
Laboratory analysis	1) SAR-COV-2 RNA quantification 2) Respiratory microbiome 3) Respiratory virome	1) SAR-COV-2 RNA quantification 2) Gastrointestinal microbiome	3) SAR-COV-2 RNA quantification 4) Immunoglobin assays 5) Absolute neutrophils/ lymphocytes/ lymphocyte subsets 6) Cytokines (TNF, Interferon, IL-1, IL-2 and IL-6)

Table 1: Plan for analysis of nasopharyngeal, fecal and blood samples

Innovation. This will be the only study to our knowledge that will correlate natural history of COVID-19 and various outcomes in patients with cancer and ***the changes in microbiome, virome, immunologic responses, and viral loads kinetics***.

References.

1. Zhu, N., et al., *A Novel Coronavirus from Patients with Pneumonia in China, 2019.* N Engl J Med, 2020. 382(8): p. 727-733.
2. Organization, W.H., *Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV).* January 30 2020.
3. Ding, Q., et al., *The clinical characteristics of pneumonia patients co-infected with 2019 novel coronavirus and influenza virus in Wuhan, China.* J Med Virol, 2020.
4. Liang, W., et al., *Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China.* Lancet Oncol, 2020. 21(3): p. 335-337.
5. Qian, G.Q., et al., *Epidemiologic and Clinical Characteristics of 91 Hospitalized Patients with COVID-19 in Zhejiang, China: A retrospective, multi-centre case series.* QJM, 2020.
6. Su, Y.J. and Y.C. Lai, *Comparison of clinical characteristics of coronavirus disease (COVID-19) and severe acute respiratory syndrome (SARS) as experienced in Taiwan.* Travel Med Infect Dis, 2020: p. 101625.
7. Wan, S., et al., *Clinical Features and Treatment of COVID-19 Patients in Northeast Chongqing.* J Med Virol, 2020.

March 26th, 2020

MD Anderson COVID-19 – SEROLOGY
TEAM (evolving): Nadim Ajami, Eleonora Dondossola, Kimberly Klein, James Kelley, Keyur Patel, Emily Yu, Fernando Martinez, Jennifer Wargo, Andy Futreal, Ignacio Wistuba

Summary

There is an urgent need to instill serology testing at our institution. Following the premise that we cannot manage what we cannot measure, it is of utmost importance to understand not only who is infected but who is still at risk and who has recovered. The direct impact of this results in our ability to care for our patients and our workforce by understanding the risks of exposure and minimizing such risk. Although we are yet to confirm the absence of reinfection or spread driven by the possible reinfection of those recovered, we can expect at least short-term immunity. Recovered and uninfected individuals could be sent out to the frontlines to continue patient care while minimizing the risk of exposure and spread.

In addition to the direct assays that have been implemented at MD Anderson that detect the virus (i.e. RT-qPCR), **we are working towards setting up serology assays that will reveal those who had the disease and recovered and could also potentially confirm infection of a person still with symptoms**. These are complementary and necessary strategies that should be available simultaneously.

To this end, this group is working on setting up two strategies that although overlapping, could be used in parallel to address throughput needs and also adapt to the varying needs observed across our institution in response to COVID19. For example, screening frontline workers and patients, and screening donated blood products among others. The first strategy is investing on rapid screening technology. A test developed by GENALYTE Inc, a company out of San Diego with whom we have been in close communication with, provides a 15-minute sample-to-answer turnaround time and is planned to be available during the first two weeks of April. GENALYTE will place one or more staffed instruments in our pathology laboratory and run assays for a cost of about $25 per sample. The second strategy is laboratory-developed test. Recognizing potential delay in implementation of the rapid screen technology, we are proposing to develop a laboratory test to identify and quantify circulating antibodies against SARS-CoV-2. This assay will be developed in collaboration with Dr. Florian Krammer from the Icahn School of Medicine at Mount Sinai who has kindly sent us reagents and protocols, and the assay will be set up together with Dr. Pedro Piedra, a world-renowned virologist that runs a CLIA lab for respiratory viruses, at Baylor College of Medicine. This with the goal of transferring a CLIA-ready SOP to our own pathology laboratory once it is set up.

The two-pronged strategy will lead to having a solution for serology testing within 30-days at MD Anderson and could be used in a complementary approach to address issues with processing throughput, confirmatory diagnosis, and redundancy in case either needs troubleshooting.

March 26th, 2020

1) Rapid Screen

Company: GENALYTE, INC (San Diego, CA)

<u>Assay</u>
Multiplexed, Rapid COVID-19 Serology Testing (15 minutes

Screen panel (available early April)
- SARS-CoV-2 surface antigens (spike and nucleocapsid protein)
  o Can evolve to include new strains (this is very important)
- Flu A&B IgM, rule out testing
- Mild CoV cases – eliminate false positive from other CoV

Instrument
- Maverick Immunoassay Analyzer (FDA cleared)
- 15-minute results
- 4 patients/run (up to 16-plex)
- 250 ul of whole blood (finger prick or venipuncture)
- Remote clinical oversight (cloud connection)

Cost:
- $55.00/sample

Strategy
- Deployed lab (instrument + personnel @ MD Anderson, operating under Genalyte's CLIA number). This will be the fastest route.
- Throughput needs: send-outs to Genalyte labs in Austin and San Diego
- Donor blood screens: send outs (24 TAT)

<u>Point persons</u>
Rob Kelley (Genalyte)
Kimberly Klein (MD Anderson)
Nadim Ajami (MD Anderson)
James Kelley (MD Anderson)
Jennifer Wargo (MD Anderson)

<u>Action Items</u>

- Set up Genalyte as a vendor (done)
- Process EAJ, work in progress

March 26th, 2020

2) Laboratory Developed Test

In collaboration with Florian Krammer (Icahn School of Medicine at Mount Sinai) and Tony Piedra (Baylor College of Medicine)

Assay
Two-step direct ELISA, using CoV-2 RBD and spike protein

Protocol: Florian Krammer (Mount Sinai), A detailed protocol for a serological assay to detect SARS-CoV-2 1 seroconversion in humans: antigen production and test setup.

Throughput:
- Screen: 760 samples/10 plates/1 run
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Action Items:

Phase I
- Complete MTAs
  o Mount Sinai <-> MD Anderson
  o MD Anderson <> Baylor College of Medicine
- Receive reagents from Mount Sinai (3/27/2020)
- Transfer reagents to Tony Piedra at BCM
- Set up/outsource antigen production and purification
- Develop RUO assay at BCM
- Transfer protocol to CLIA (BCM)

Timeline: 1 month
Budget: Protein purification and expression, ~$10,000

Phase II
- Transfer SOP to MD Anderson
- In-house validation
-
Timeline: TBD
Budget: Assay validation, TBD

March 26th, 2020

March 26th, Meeting Minutes

<u>GENALYTE</u>
- CLIA regulation, GENALYTE
- Figure out logistics (In-house (20-min), vs. lab in Austin/reference (24 hrs.))
- Reagent rental (11 machines available)

<u>Other tech</u>
- Roche and other players likely to come out with rapid assays

<u>Protocol development</u>
- Lab protocol, waiver consent

<u>Life Sciences Plaza</u>
- R&D (Translational Molecular Pathology lab facility)
  o RT-qPCR, improve throughput
  o Can we add serology

March 26th, 2020

MD Anderson COVID-19 – SEROLOGY
TEAM (evolving): Nadim Ajami, Eleonora Dondossola, Kimberly Klein, James Kelley, Keyur Patel, Emily Yu, Fernando Martinez, Jennifer Wargo, Andy Futreal, Ignacio Wistuba

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Cost:
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Strategy
- Deployed lab (instrument + personnel @ MD Anderson)
- Send-outs to Genalyte labs in Austin and San Diego
- Donor blood screens: send outs (24 TAT)

Throughput:
- 3,000 tests/month (in-house + send outs)

<u>Point persons</u>
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Kimberly Klein (MD Anderson)
Nadim Ajami (MD Anderson)
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March 26th, 2020

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- Transfer SOP to MD Anderson
- In-house validation
-
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Budget: Assay validation, TBD

March 26th, 2020

MEETING SUMMARY

March 26th

GENALYTE
- CLIA regulation, GENALYTE
- Figure out logistics (In-house (20-min), vs. lab in Austin/reference (24 hrs.))
- Reagent rental (11 machines available)

Other tech
- Roche and other players likely to come out with rapid assays.

Protocol development
- Lab protocol, waiver consent

Life Sciences Plaza
- R&D (Translational Molecular Pathology lab facility)
  o RT-qPCR, improve throughput
  o Can we add serology testing through TMP

End.

March 27th

Call with Genalyte

Ignacio Wistuba -> Ferran Prat (review and expedite agreement)
Samples -> Austin
Minimum volume commitment, 3000/samples
Launch assay, April 8th
Cost $55.00/sample
Instrument capacity: 288 samples/day (24hr operation)
- 4 patients per run, 3 shifts
- Ever 20min, new run
- 1 operator per shift
- Whole blood (EDTA, 200 uls/sample), or serum

Add terminology in the contract to include Austin laboratory send-outs
Are there additional instruments to be deployed on-site
Instrument + assay, validated and approved FDA
COVID19 assay, pending approval
Under COVID19 response, instruments and tests can be deployed (Section D, FDA guidance).
LDTs can be sent to MD Anderson

March 26th, 2020

Get Genalyte the exact location of the room for the CLIA license
Ferran -> Genalyte (Rob)

33 Serology Tests Approved by the FDA as of 08/07/2020

- Chemiluminescent immunoassay (CLIA/CMIA)
- Fluorescent microsphere immunoassay (FMIA)
- Multiplex immunoassay (MIA)
- Lateral Flow
- ELISA

Membrane glycoprotein (M)

Nucleocapsid protein (N)

Abbot Architect SARS-CoV-2 IgG

Roche Elecsys Anti-SARS-CoV-2 (Pan-Ig)

Spike protein (S)

Mount Sinai Hospital Laboratory COVID19 ELISA
SPIKE+RBD, adapted for IgG + IgM testing

Envelope protein (E)

RNA

APPROVED ASSAYS	
SPIKE	20
NUCLEOCAPSID	7
SPIKE + NC	6

Study Framework

1. Target population	Non-patient facing research employees only
2. Method of recruitment	e-mail from CSO; website offering opportunities to participate in research.
3. Method of testing	Quantitative or semi-quantitative (ordinal)
4. Labs for testing	Labs of collaborators: Wargo, Yee, Kalluri, Molldrem
5. Sharing with participants	No sharing of individual results. Aggregate dissemination of research findings to research community (eg. via a Town Hall format) and scientific publications.
6. Funding source/ impact	Minimal FTE involvement of Employee Health in 1MC, where we propose to conduct blood draws and conduct on-site consent to study participation. For pilot study, will repurpose research staff in collaborators' labs to devote effort to administration of questionnaire, sample transfer, data organization, and molecular assays. Employee Health will perform sample collection for ~250 study participants; the Office of the CSO will provide funds for collection tubes to Employee Health.
7. Timeline for protocol	1 week (14-May-2020)

COVID19-ResearchTF Diagnostics and serology

Co-leaders: Hector Alvarez , James Kelley, Nadim Ajami

Florencia McAllister, Eleonora Dondossola, Raghu Kalluri

Research Questions

- Type and duration of immunity observed in cancer patients with COVID19
    - Ab production timeline (IgM, IgG, subclasses) in the context of HLA loci.
    - Neutralizing antibodies (how can we test outside of BSL3 lab)
    - Immune correlates of disease severity (CRS, pro-inflammatory macrophages, granulocytes)
    - Lymphocytopenia – measure T/B cell responses

- Correlation between symptoms, antigen, antibodies in cancer patients

Critical for:
- Strategic clinical management
- Evaluation of the efficacy of vaccination in different individuals in the general population
- Assignment of clinical professional and managerial teams amid interactions with COVID-19 patients.

What we know (2 slides)

- Insert summary text/figures from slides 9-12 (Wolfel et al, Nature; Bao, BioarXiv, 2020)

- Antibodies -> disease severity (Li Liu JCI Insight, 2019)

Efforts

- MD Anderson ELISA (Raghu Kalluri)

- ELISA - LDT (Tony Piedra, BCM)
  - Protocol and reagents obtained from Florian Krammer (Icahn School of Medicine, Mount Sinai).
  - Results: RBD/mAb and polyclonal sera (EXAPAND)

- PoC (Kathleen Schmeler, MD Anderson & Rebecca Richards-Kortum, Rice University)
  - Group has experience in developing PoC for viral antigen and antibody detection for low-resource settings

Next Steps

- Sample sources
  - APOLLO protocol
- ELISAs
- Others

SARS-CoV-2 localization

- Throat-derived samples: present, infectious virus was readily isolated

- Lung-derived samples: present, infectious virus was readily isolated

- Stool: present, not infectious in spite of high virus RNA concentration

- Blood: absent (n=0/31)

- Urine: absent (n=0/27)

Day 1-7: Virus was readily isolated in 16.66% of swabs and 83.33% of sputum samples
> Day 8: no isolates in spite of ongoing high viral loads

Virus isolation success also depended on viral load: samples containing <10^6 copies/mL (or copies per sample) never yielded an isolate.

Wolfel et al., Nature, April 1, 2020

Type of immunity observed in cancer patients

Duration of immunity

Neutralizing antibodies (how can we test outside of BSL3 lab)

Antigenemia, viremia?

Correlation between symptoms, antigen, antibodies (disease course). Is this different for cancer patients?

Workforce immunity

Immunity passport (COVID19-Immune wristband)

SARS-CoV-2 detection by RT-PCR

Sputum vs. swab have overall comparable viral load

Oro- or naso-pharyngeal SWAB
There were **no differences** in viral loads or detection rates when comparing naso- vs. oropharyngeal swabs

Earliest swab taken day 1 of symptoms (very mild or prodromal)

Day 1-5
All swabs from all patients tested **positive**
(average virus RNA load 6.76×10^5 copies/whole swab until day 5)

> Day 5
Average viral load of 3.44×10^5 copies per swab and a detection rate of 39.93%.
The last positive-testing swab sample was taken on day **28 post-onset**.

SPUTUM
Average viral load in sputum was 7.00×10^6 copies per mL

Virological assessment and seroconversion of hospitalized patients with COVID-2019

Fig. 2 | Viral load kinetics, seroconversion and clinical observations in individual cases. Panels A to I correspond to cases #1, #2, #3, #4, #7, #8, #10, #14, and #16 in Böhmer et al.[11] Dotted lines, limit of quantification. Experiments were performed in duplicate and the data presented are means of results obtained by two laboratories independently.

Seroconversion in patients with COVID-2019

Seroconversion was detected by **IgG** and **IgM** immunofluorescence and occurred by **7 days** in **50% of patients** (**14 days** in **all**). No viruses were isolated after day 7. The titer of antibodies did not correlate with clinical course.

Wolfel et al., Nature, April 1, 2020

SARS-CoV-2 **IgA, IgM** and **IgG** response:

N=208 (from 82 confirmed + 58 probable cases with qPCR negative but had typical manifestation; 41 samples 1–7days; 84 samples 8 –14 days; 83 samples > 14 days PSO)

IgM detected as soon as day 1; increased until day 14 PSO and remained stable.

IgGs detected 14 days PSO, increased up to day 21 PSO and remained stable. In confirmed and probable cases, the positive rates of IgM antibodies were 75.6% and 93.1%, respectively.

Before day 5.5:
Detection rate by qPCR > than IgM ELISA assay before 5.5 days PSO
After day 5.5:
Detection rate by IgM ELISA > qPCR method.
The positive detection rate is only 51.9% as single PCR test, but significantly increased (98.6%) when applied IgM ELISA assay to PCR-negative patients

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
IgM	1	6	3	3	7	3	3	9	10	13	9	18	11	13	14	12	7	7	8	3	1	3	2	3	4
IgG	1	8	3	5	5	1	4	2	8	10	8	16	12	13	12	11	6	7	8	2	1	4	2	3	4
PCR	1	8	3	5	11	4	6	4	9	1	3	5	8	4	8	2	5	6	6	2	0	2	0	1	1
Total	1	8	3	5	11	7	6	10	12	13	10	21	13	13	15	12	8	7	9	4	1	4	2	3	4

Guo, Clinical Infectious Diseases, 2020

No cross-reactivity of SARS-CoV-2 rNP with human plasma positive for IgG antibodies against NL63, 229E, OC43, and HKU1 **coronaviruses**

Guo, Clinical Infectious Diseases, 2020;Amanat, MedrXiv, 2020

Reinfection could not occur in SARS-CoV-2 infected rhesus macaques

Monkeys infected ---> Symptoms (weight loss, viral replication mainly in nose, pharynx, lung and gut, as well as moderate interstitial pneumonia at 7 days post-infection) disappeared ---> seroconversion ---> re-challenge ½ of the monkeys

No viral loads in nasopharyngeal and anal swabs
No viral replication in all primary tissue compartments at 5 days post-reinfection was found in re-exposed monkeys.

Bao, BioarXiv, 2020

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Duration of immunity

Neutralizing antibodies (how can we test outside of BSL3 lab)

Antigenemia, viremia?

Correlation between symptoms, antigen, antibodies (disease course). Is this different for cancer patients?

Workforce immunity

Immunity passport (COVID19-Immune wristband)

Countries who plan to set up an immunity certificate

UK https://economictimes.indiatimes.com/news/politics-and-nation/immunity-certificates-the-passport-to-a-new-life-after-coronavirus/articleshow/74963050.cms?from=mdr
https://www.nytimes.com/reuters/2020/04/02/world/europe/02reuters-health-coronavirus-britain-immunity.html

Germany https://www.newsweek.com/germany-antibodies-tests-general-public-immunity-certificates-1494934

Italy https://www.nytimes.com/2020/04/04/world/europe/italy-coronavirus-antibodies.html

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  - Protocol and reagents obtained from Florian Krammer (Icahn School of Medicine, Mount Sinai).
  - Results: RBD/mAb and polyclonal sera (EXAPAND)

- PoC (Kathleen Schmeler, MD Anderson & Rebecca Richards-Kortum, Rice University)
  - Group has experience in developing PoC for viral antigen and antibody detection for low-resource settings

Next Steps

- Sample sources
  - APOLLO protocol
- ELISAs
- Others

SARS-CoV-2 localization

- Throat-derived samples: present, infectious virus was readily isolated

- Lung-derived samples: present, infectious virus was readily isolated

- Stool: present, not infectious in spite of high virus RNA concentration

- Blood: absent (n=0/31)

- Urine: absent (n=0/27)

Day 1-7: Virus was readily isolated in 16.66% of swabs and 83.33% of sputum samples
> Day 8: no isolates in spite of ongoing high viral loads

Virus isolation success also depended on viral load: samples containing <10^6 copies/mL (or copies per sample) never yielded an isolate.

Wolfel et al., Nature, April 1, 2020

Type of immunity observed in cancer patients

Duration of immunity

Neutralizing antibodies (how can we test outside of BSL3 lab)

Antigenemia, viremia?

Correlation between symptoms, antigen, antibodies (disease course). Is this different for cancer patients?

Workforce immunity

Immunity passport (COVID19-Immune wristband)

SARS-CoV-2 detection by RT-PCR

Sputum vs. swab have overall comparable viral load

Oro- or naso-pharyngeal SWAB

There were **no differences** in viral loads or detection rates when comparing naso- vs. oropharyngeal swabs

Earliest swab taken day 1 of symptoms (very mild or prodromal)

Day 1-5

All swabs from all patients tested **positive**
(average virus RNA load 6.76×10^5 copies/whole swab until day 5)

> Day 5

Average viral load of 3.44×10^5 copies per swab and a detection rate of 39.93%.
The last positive-testing swab sample was taken on day **28 post-onset**.

SPUTUM
Average viral load in sputum was 7.00×10^6 copies per mL

Virological assessment and seroconversion of hospitalized patients with COVID-2019

Fig. 2 | Viral load kinetics, seroconversion and clinical observations in individual cases. Panels A to I correspond to cases #1, #2, #3, #4, #7, #8, #10, #14, and #16 in Böhmer et al.[11] Dotted lines, limit of quantification. Experiments were performed in duplicate and the data presented are means of results obtained by two laboratories independently.

Wolfel et al., Nature, April 1, 2020

Seroconversion in patients with COVID-2019

Seroconversion was detected by **IgG** and **IgM** immunofluorescence and occurred by **7 days** in **50% of patients** (**14 days** in **all**). No viruses were isolated after day 7. The titer of antibodies did not correlate with clinical course.

Wolfel et al., Nature, April 1, 2020

SARS-CoV-2 **IgA, IgM** and **IgG** response:

N=208 (from 82 confirmed + 58 probable cases with qPCR negative but had typical manifestation; 41 samples 1–7days; 84 samples 8 –14 days; 83 samples > 14 days PSO)

IgM detected as soon as day 1; increased until day 14 PSO and remained stable.

IgGs detected 14 days PSO, increased up to day 21 PSO and remained stable. In confirmed and probable cases, the positive rates of IgM antibodies were 75.6% and 93.1%, respectively.

Before day 5.5:
Detection rate by qPCR > than IgM ELISA assay before 5.5 days PSO
After day 5.5:
Detection rate by IgM ELISA > qPCR method.
The positive detection rate is only 51.9% as single PCR test, but significantly increased (98.6%) when applied IgM ELISA assay to PCR-negative patients

Guo, Clinical Infectious Diseases, 2020

No cross-reactivity of SARS-CoV-2 rNP with human plasma positive for IgG antibodies against NL63, 229E, OC43, and HKU1 **coronaviruses**

Guo, Clinical Infectious Diseases, 2020;Amanat, MedrXiv, 2020

Reinfection could not occur in SARS-CoV-2 infected rhesus macaques

Monkeys infected ---> Symptoms (weight loss, viral replication mainly in nose, pharynx, lung and gut, as well as moderate interstitial pneumonia at 7 days post-infection) disappeared ---> seroconversion ---> re-challenge ½ of the monkeys

No viral loads in nasopharyngeal and anal swabs
No viral replication in all primary tissue compartments at 5 days post-reinfection was found in re-exposed monkeys.

Bao, BioarXiv, 2020

Type of immunity observed in cancer patients

Duration of immunity

Neutralizing antibodies (how can we test outside of BSL3 lab)

Antigenemia, viremia?

Correlation between symptoms, antigen, antibodies (disease course). Is this different for cancer patients?

Workforce immunity

Immunity passport (COVID19-Immune wristband)

Countries who plan to set up an immunity certificate

UK https://economictimes.indiatimes.com/news/politics-and-nation/immunity-certificates-the-passport-to-a-new-life-after-coronavirus/articleshow/74963050.cms?from=mdr
https://www.nytimes.com/reuters/2020/04/02/world/europe/02reuters-health-coronavirus-britain-immunity.html

Germany https://www.newsweek.com/germany-antibodies-tests-general-public-immunity-certificates-1494934

Italy https://www.nytimes.com/2020/04/04/world/europe/italy-coronavirus-antibodies.html

MATERIALS
- Expi293 kit (Gibco # A14635) $2170
- Expi293 Expression Medium (Gibco # A1435102) 2L, $262.00/L-> $524
- ExpiFectamineTM 293 Transfection Kit (GIbco #A14524) $726/L -> $1425
- Ni-NTA Agarose (Qiagen #30230 or equivalent) $1114
- SDS-PAGE gels (Bio-Rad #4561094 or equivalent) $119
- Sodium phosphate monobasic monohydrate $NaH_2PO_4. H_2O$ (Sigma Aldrich #S3522 or equivalent); Sodium Chloride NaCl (Sigma-Aldrich #S3014 or equivalent); Imidazole (Sigma-Aldrich #I5513 or equivalent) $ 770
- Stericup Quick Release-GP Sterile Vacuum Filtration System (MilliporeSigma S2GPU05RE or equivalent) $ 195
- Disposable Polycarbonate Erlenmeyer Flasks (Corning #431147) 10, $559
- 5mL Polypropylene columns (Qiagen #34964 or equivalent) $189
- AmiconTM Ultra Centrifugal Filter Units 10 kDa (MilliporeSigma #UFC901024 or equivalent) $305
- AmiconTM Ultra Centrifugal Filter Units 50 kDa (MilliporeSigma #UFC905024 or equivalent) $305
- Flat-Bottom Immuno Nonsterile 96-Well Plates 4 HBX (Thermo Scientific #3855, or equivalent) $209
- Flat Bottom Cell Culture Plates (Corning #3599 or equivalent) $293
- Milk Powder (AmericanBio #AB10109-01000, or equivalent) $83
- Phosphate Buffered Saline (10X) (CorningTM 46013CM or equivalent) $320
- Sterile reservoirs (Fisher Scientific #07-200-127 or equivalent) $200
- Anti-Human IgG (Fab specific)-Peroxidase antibody produced in goat (Sigma #A0293) $372
- Sigma OPDFast platelets (Sigma #P9187) $414

TOT $9570 + $430 plasticware = $10000

NO COST
- Opti-MEMTM I Reduced Serum Medium (Gibco #31985088)
- PBS (1X) (Gibco #10010-023 or equivalent)
- SDS-PAGE cell and power supply
- Polypropylene sterile conical tubes
  - 15 mL (Denville Scientific #C1018P or equivalent)
  - 50 mL (Fisher Denville Scientific #C1060P or equivalent)
- Micropipette tips
  - 20 µL barrier tips (Denville Scientific #P1121 or equivalent)
  - 200 µL barrier tips (Denville Scientific #P1122 or equivalent) ○ 200 µL tips (USA Scientific #1111-1700 or equivalent)
  - 1000 µL barrier tips (Denville Scientific #P1126 or equivalent)
- Sterile, serological pipettes
  - 5mL (Falcon #356543 or equivalent) ○ 10mL (Falcon #357551 or equivalent) ○ 25 mL (Falcon #357535 or equivalent) ○ 50 mL (Falcon #356550 or equivalent)
- 1.5 mL Eppendorf tubes (Denville #C2170 or equivalent)
- Pipet-Aid
- Micropipettes
- Class II biological safety cabinet
- Trypan blue solution, 0.4 % (Gibco #15250-06 or equivalent)

- Cell counting slides (Invitrogen #C10312 or equivalent)
- Countess II cell counter or equivalent
- ==*CO_2 incubator with built in shaker*== (Eppendorf New Brunswick S41i or Equivalent)
- Benchtop shaker (Benchmark #BT3000 or equivalent)
- Cooling Centrifuge (Eppendorf 5810R or equivalent)
- Refrigerator at 4°C (+/- 1°C)
- Ultra-Low Freezer (-80°C)

EVALUATING THE SEROPREVALENCE OF SARS-COV-2 ANTIBODIES IN AN ASYMPTOMATIC POPULATION AT MD ANDERSON CANCER CENTER

RESEARCH PROTOCOL

Leads: Eleonora Dondossola and Nadim Ajami

Internal Collaborators: Giannicola Genovese, Jennifer Wargo, Pam Sharma,

External Collaborators: Tony Piedra (BCM), Maria Elena Bottazzi (BCM), Florian Krammer (Mount Sinai), Michael Laposata (UTMB)

(UNIVERSAL) REAGENTS

Antigens and Antibodies:

- **Receptor Binding Domain of SARS-CoV-2 (NR-52306)**
    - Plasmid grown and RBD purified in-house
    - Antigen ordered from BEI Resources (ordered, limited quantity)
    - Antigen sourced from BCM (Maria Elena Bottazzi, MTA wip)

- **Spike protein of SARS-CoV-2 (NR-52306)**
    - Plasmid grown and RBD purified in-house (ramping-up)
    - Antigen ordered from BEI Resources (ordered, limited quantity)

- **Goat Anti-Human IgG HRP (Sigma)**
- **Goat Anti-Human IgM HRP (Sigma)**
- **Rabbit SARS-CoV-2 S1 mAb (Sino Biological)**

ASSAY SETUP

RBD-SARS-CoV-2
- BEI Resources
- Maria Elena Bottazzi (BCM)
- Eleonora Dondossola (MDACC)
- Gianni Genovese (MDACC)

POSITIVE SERA
- ITB MDACC
- Pathology, UTMB
- Tony Piedra, BCM

SPIKE-SARS-CoV-2
- BEI Resources
- Gianni Genovese (MDACC)
- Eleonora Dondossola (MDACC)

PREPANDEMIC SERA
- Jen Wargo
- Tony Piedra, BCM
- Pathology, UTMB

ASSAY UTILIZATION

SCREEN ELISA (RBD)

Day1
- Precoated and blocked plate
- Heat inactivate samples
- Predilute samples
- Sample dilution
- Secondary Ab
- Develop

Throughput: 1 operator, 760 samples/10 plates per run

CONFIRMATORY ELISA (SPIKE)

Day1
- Precoated and blocked plate
- Block
- Predilute samples
- Serial dilutions
- Secondary Ab
- Develop

1 operator, 140 samples/10 plates per run

TIMELINE & ACTIVITES

Week	Apr 27	May 4	May 11	May 18	May 25	June 1	June 8
REAGENTS SOURCING	███	███	███				
ELISA SETUP		███	███	███	███		
ELISA VALIDATION					███	███	
TESTING							███

LAB	ACTIVITY
JEN WARGO (MDACC)	Plasmid amplification & protein expression (RBD and spike)
ELEONORA DONDOSSOLA (MDACC)	Protein purification & ELISA Setup
GIANNI GENOVESE (MDACC)	Plasmid amplification (RBD and spike)
MARIA ELENA BOTTAZZI (BCM)	RBD
TONY PIEDRA (BCM)	Protocol details
MICHAEL LAPOSATA (UTMB)	Positive and pre-pandemic sera

COVID19 SEROPREVALENCE RESEARCH STUDY

PHASE I	PHASE II	PHASE III

ASSAY DEVELOPMENT
4 weeks

ASSAY UTILIZATION
Start in 4 weeks, in course as permitted

ASSAY EXPANSION
To be determined

BCM/MDA – ELISA
- Quantitative
- Antigen: Receptor Binding Domain (screening), and Spike protein (confirmatory)
- Antibody: IgG and IgM
- Controls: Positive sera and mAb/polyclonal sera

SET 1

COVID19 confirmed and probable cases – samples banked at ITB

SET 2

Research, healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

COVID19 ELISA → **Positive** → Presumed Immune

Negative ↓

Not presumed immune

IgM + ; IgG -   Resolving infection

IgM + ; IgG +   Convalescent

IgM - ; IgG +   Recovered

- Expansion to wider population

- Repeat testing to determine duration of antibodies in circulation

- Development of neutralization assays

END CoV-2: Evaluation of a Novel Point-of-care Diagnostic Test for SARS-CoV-2

Co-Principal Investigators:
- MD Anderson: K. Schmeler, MD (Gynecologic Oncology); K. Patel, MD, PhD (Hematopathology)
- Rice University: R. Richards-Kortum, PhD (Bioengineering)

Collaborators:
- MD Anderson: I. Wistuba, MD (Pathology & Laboratory Medicine); Bryan Fellman, MS (Biostatstics); X. Han, MD (Laboratory Medicine)
- Rice University: K. Kundrod, M. Natoli, C. Smith, M. Chang (Bioengineering)

Primary Objective: To evaluate the clinical performance of a novel point-of-care (POC) diagnostic test for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Secondary Objectives: 1) To compare the clinical performance of provider-collected nasopharyngeal samples with self-collected nasal swab, cheek swab and oral rinse samples using the novel SARS-CoV-2 diagnostic test; 2) To measure viral load and evaluate the role of viral load in COVID-19 severity.

Background and Rationale: There is an urgent need for widespread access to accurate, affordable diagnostic tools to detect SARS-CoV-2, the virus that causes COVID-19. Dr. Rebecca Richards-Kortum and team at Rice University have developed a novel POC, self-contained nucleic acid amplification test (NAAT) that fully integrates sample preparation, isothermal reverse transcription and amplification, and real-time detection into a simple and streamlined workflow. The total instrument cost is <$5,000, tests are performed at POC without the need for expensive laboratory set up or highly skilled laboratory personnel and, results are available within 30-45 minutes, and the test does not required highly skilled laboratory technicians to run. Our collaborative team of investigators from Rice University and MD Anderson have extensive experience working together to develop and evaluate similar human papillomavirus (HPV) tests for cervical cancer screening in low resource settings in the US (Lyndon B. Johnson (LBJ) Hospital, Rio Grande Valley) and globally (Latin America and Africa). Given the innovative design, affordability and fast turn-around-time of this novel SARS-CoV-2 POC test, we believe it will have utility in both high-resource settings as well as rural and medically underserved areas in the US and globally.

Inclusion Criteria: 1) Age ≥ 18; 2) Qualifies for SARS-CoV-2 testing at MD Anderson testing sites (may include MD Anderson patients and employees) according to institutional criteria at time of enrollment; 3) Signed informed consent and ability to perform protocol-required activities.

Study Design: This is a cross-sectional study that will enroll up to 1,500 participants presenting for SARS-CoV-2 testing at MD Anderson testing sites. After obtaining informed consent, we will collect information including demographics, smoking/vaping history, symptom burden and underlying medical conditions. Two nasopharyngeal swabs will be collected by a health care provider, followed by self-collection of a nasal swab, cheek swab and oral rinse sample. One of the nasopharyngeal swabs will be sent for standard-of-care SARS-CoV-2 testing at the MD Anderson Molecular Diagnostic Lab by RT-PCR or a suitable substitute reference method. The second nasopharyngeal swab, as well as the self-collected nasal swab, cheek swab and oral rinse samples will be transferred to Rice University for testing on the novel diagnostic platform. Participants will complete two questionnaires sent via email or text message using REDCap. The first will solicit feedback regarding the provider-collected vs. self-collected samples and the second will include questions regarding disease severity and outcomes.

Analysis: We will determine the sensitivity and specificity of the Rice test compared with the standard of care test for provider-collected nasopharyngeal samples. In addition, we will compare the performance of self-collected nasal, cheek and oral rinse samples. We will also evaluate the role of viral load in determining COVID-19 severity.

Please see attached protocol for full details.

Coronavirus Structure

Envelope protein

Spike glycoprotein

Membrane glycoprotein

RNA
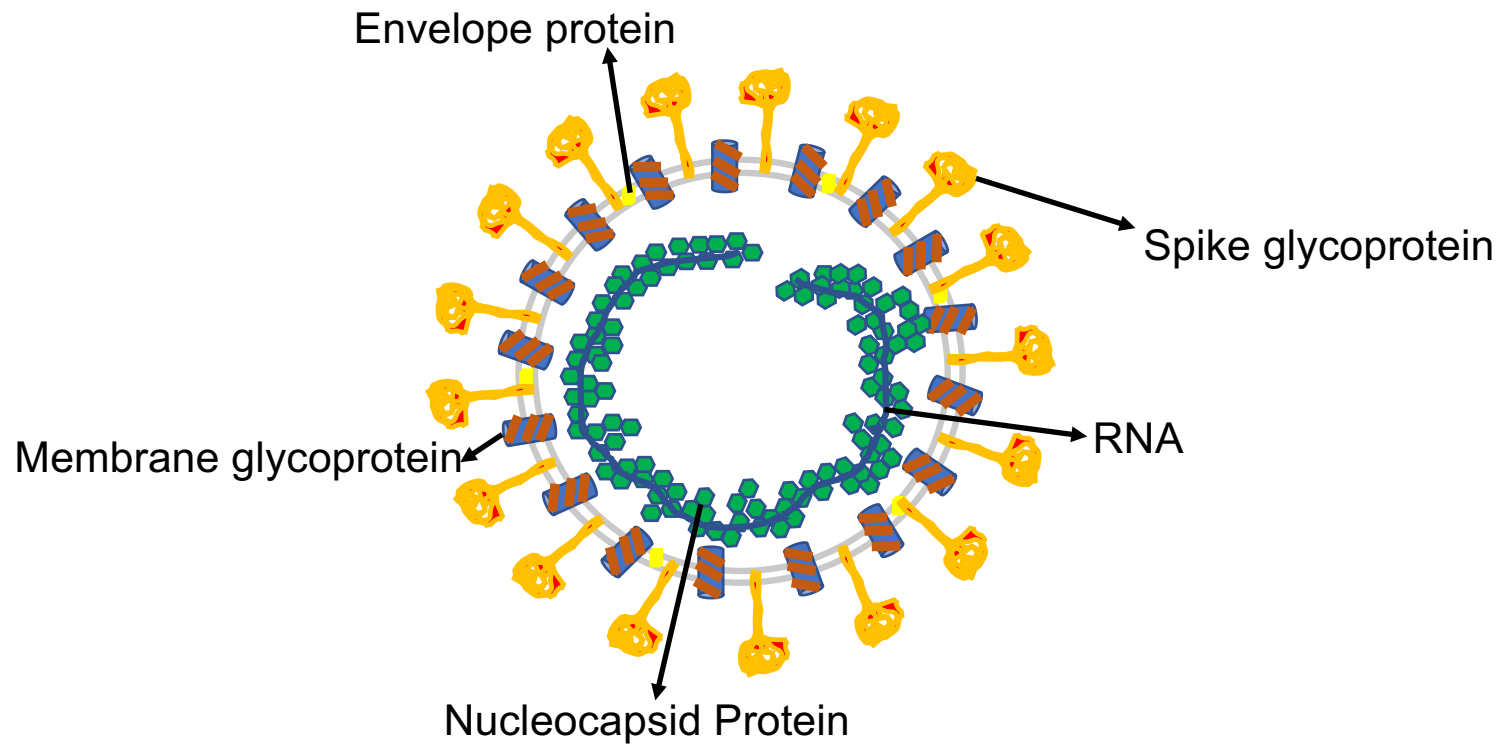
Nucleocapsid Protein

Serology test detect anti-spike protein IgM and IgG (MDA-SC2S serology test)

Goal: ELISA test to detect IgM and IgG in human plasma/serum against SARS-CoV-2 spike protein.

Reagents: Recombinant soluble SARS-CoV-2 Spike ectodomain protein production in 293T cells was achieved and based on the yields, we can easily conduct 1000 research trial tests a day and with the ability to scale it up further.

Resources: For 1000 research trial tests tests a day, we will need 5 individuals and a supervisor for production, testing and data reporting.

Equipment and supplies: We already have three ELISA plate readers and all the other essential supplies for the trail runs. In the long term, we will require the ability to order supplies.

Estimated cost: $2 per test, and all the infrastructure and personnel costs. We do not require any additional funding at this time point.
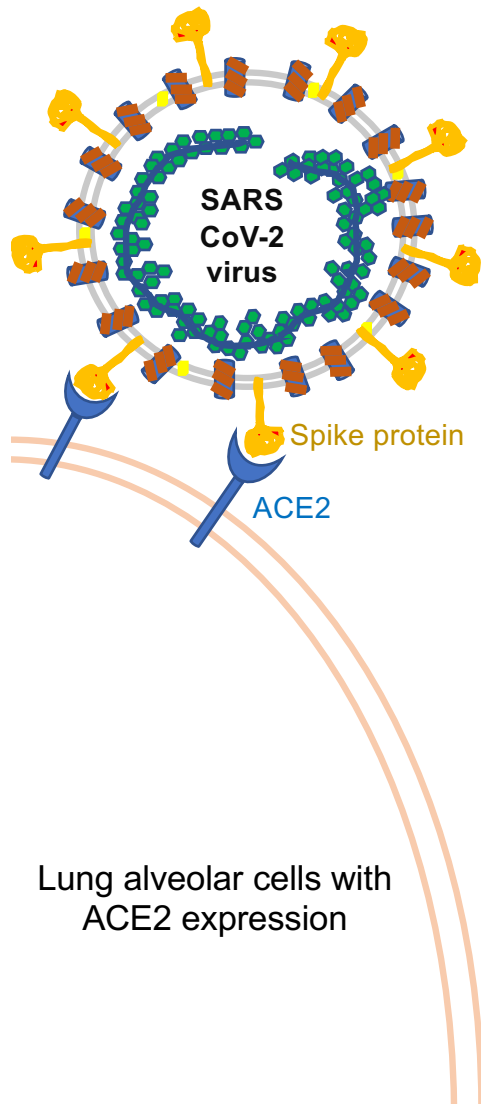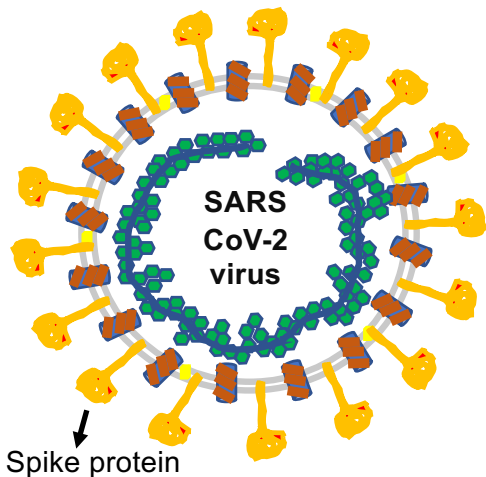
Production of soluble recombinant SARS-CoV-2 spike ectodomain protein (rSC2SE protein)

Goal: 293T cells were transfected with plasmid for expression of soluble recombinant SARS-CoV-2 spike ectodomain protein with strep 2X and 6X his-tag (rSC2SE protein). The culture supernatant was harvested and protein isolated using his-tag column employing HPLC. We were able to generate 200 micrograms of rSC2S protein per 20 ml of culture supernatant. This amount of rSC2SE protein is enough to perform 2000 serology tests using human plasma/serum. We can easily scale it up to about 1 mg of purified protein a day.

SARS CoV-2 virus

Spike protein

A

SS 1
NTD
RBD
SD1
SD2
S1/S2
S2′
FP
HR1
CH
CD
HR2 CT
TM
1208
1273

rSC2SE protein

+ 293T cells
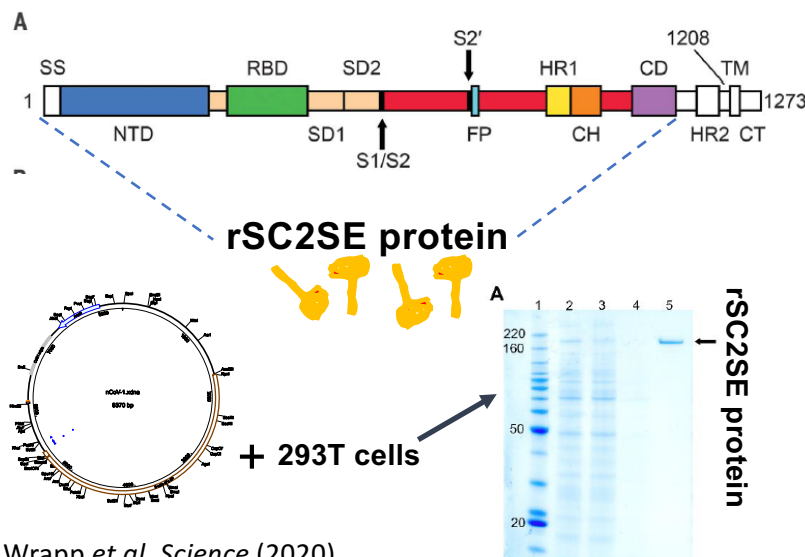
Wrapp *et al. Science* (2020)
Kalluri Laboratory (2020)

rSC2SE protein

220
160
50
20

1 2 3 4 5

A

mAU
26.58

UV 1_280
Cond
Conc B

Peak Elution Volume = 141.8 mL

141.84

Fractions
A2 A3 A4 A5 B1 B2 B3

Input [1:1000]
FT [1:1000]
A2 [1:100]
A3 [1:100]
A4 [1:100]
A5 [1:100]
B1 [1:100]
B2 [1:100]
B3 [1:100]
L

250
150
75
50
37
25
15
10
kDa

← Spike$_{ECTO}$

SDS-PAGE

4-12% SDS-PAGE
Simply Blue Stain (CBB)
Reducing (DTT)

Controls
L FT   B1 B2 B3

250
150
75
50
37
25
20
15
10
kDa

← Spike$_{ECTO}$

Western Blot

4-12% Reducing SDS-PAGE
aStrepTactin mAb 1:1000
anti-Mouse HRP 1:4000

Size Exclusion Chromatography
Column: Superose 6 (10/300) SEC
Mobile Phase: PBS
Sample: 100 uL of 10 mL Pooled NiNTA Fractions (A2-B3)

A280
Time

Spike$_{ECTO}$

Schematic illustration of the MDA-SC2S Serology Test

SARS CoV-2 virus
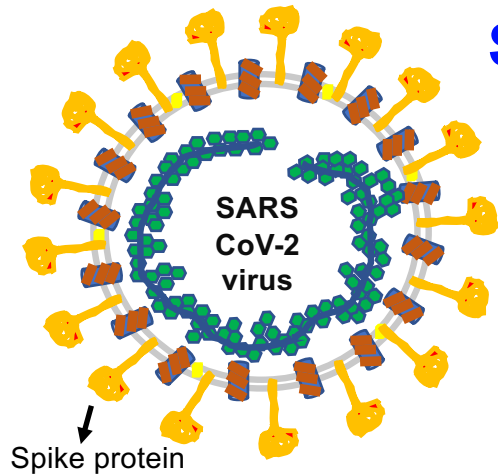
Spike protein

In direct Enzyme-linked immunosorbent Assay (ELISA)

Substrate

Secondary Antibody Conjugate

Primary Antibody

Ag

Indirect Assay
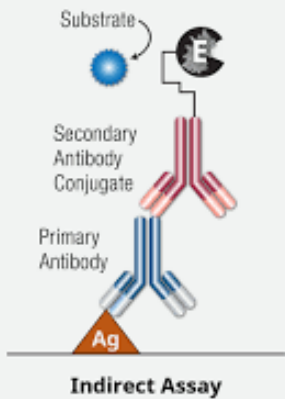
Spike protein ectodomain

Blocking of remaining active sites (BSA, Caesin, etc)

Primary anti-spike antibodies, controls and human serum/plasma

The entire assay can be performed in ~2 hours including data reporting

Provide enzyme substrate

3X Wash to remove unbound excess reagents

3X Wash to remove unbound excess reagents

Anti-human IgM/IgG antibodies with enzyme probe

MD Anderson COVID-19 Task Force

Steering Committee: Giulio Draetta, CSO (Chair), Ignacio Wistuba, Patrick Hwu.

Facilitators: Anirban Maitra and Scott Kopetz

Goal:
  (1) To identify high priority areas of clinical and translational research relevance germane to COVID-19 in cancer patients
  (2) To develop immediate, intermediate and long term timelines for implementation, so as to position MDACC as a national leader on COVID-19 research
  (3) To form interdisciplinary working groups for each high priority area with inclusiveness and transparency

High Impact Areas (working groups):

1: Diagnostics and serology
Co-leaders: Hector Alvarez and James Kelley

2: Immunobiology of COVID-19 in cancer
Co-leaders: Pam Sharma and Jen Wargo

3: Experimental therapeutics (preclinical)
Co-leaders: Raghu Kalluri and Jason Bock

4: Cellular therapies
Co-leaders: Katy Rezvani and Cassian Yee

5: COVID-19 Data science
Co-leaders: David Jaffray and Andy Futreal

6: Clinical therapeutics of COVID-19
Co-leaders: Ana Aparicio and Jennifer Litton

7: Epidemiology (including genetic predisposition) and community health (including apps)
Co-leaders: Paul Scheet and Lorna McNeil

8: Viral pathogenesis and virology
Co-leaders: Shao-Cong Sun, Nadim Ajami, Samuel Shelburne

Participation in each working group will occur via open solicitation and specific demonstration of capabilities to contribute to the mission of the WG (for example, access to BSL-3 facility or reagents pertinent to COVID-19 research or access to

specific expertise to further the goal of the WG). WG leads will have a say on capping the number of faculty participants within a specific WG.

Phase 1: Longitudinal biospecimen collection and data science
Timeline immediate
Tasks:

IRB for collection
Collection and storage specifications (volume, tubes, immediate processing needs)
Putting in place CDEs for EPIC
ANY WG activities (writing clinical protocols, INDs for repurposed drugs, IRBs, *in silico* research) should be considered as Phase 1

Phase 2: Analysis of Phase 1 biospecimens
Timeline: Beginning in ~3 weeks and onwards
Tasks:

Define usage parameters
(WG 1 and 2 will be high end users)
Define personnel needs
Define laboratory space distinct from clinical space

Phase 3: Implementation of limited wet bench research opportunities
Timeline: To be evaluated by MDACC President/CSO (optimistically end April to early May)
Tasks:

Identify restricted laboratory space (including as needed BSL-3 space) and personnel
Obtain internal funding – mechanism to be decided
(Multiple NIH supplements and de novo NIH/NSF/DOD grants are open for COVID as well)
Repurposed therapeutic opportunities should begin in Phase 2-3

Phase 4: Return to full capacity
Timeline: end summer 2020
Tasks:

Position platforms (especially IACS and ORBIT) for therapeutic development
Partner with Molecular Diagnostics and Microbiology of clinical grade implementation of research assays
Consider lead-in safety trials for experimental agents developed in earlier phases.

ADDITIONAL RESOURCES:
Revised CDC Guidelines on BSL-3 and BSL-2 facilities for COVID-19 research
https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html

COVID-19 curated literature on Box (Created by Cassian Yee)
https://mdacc.box.com/s/qpz1krv33kfhqdsw4qql32qo8rjcqgk7

COVID-19 Funding opportunities (From Johns Hopkins University)
https://hub.jhu.edu/novel-coronavirus-information/research-preparedness/research-preparedness-covid-19-funding-opportunities/

Viral load

Days since infection

1 2 3 4 5 6 7 8 9 10 11 12 13 14
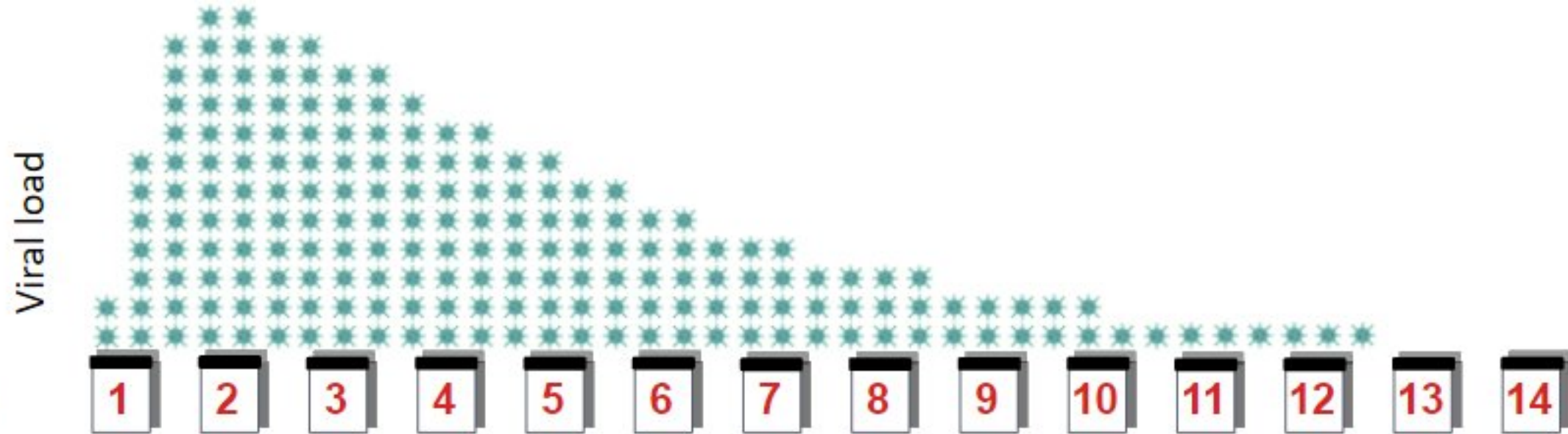
To stop the spread, find these people

Asymptomatic

Symptomatic
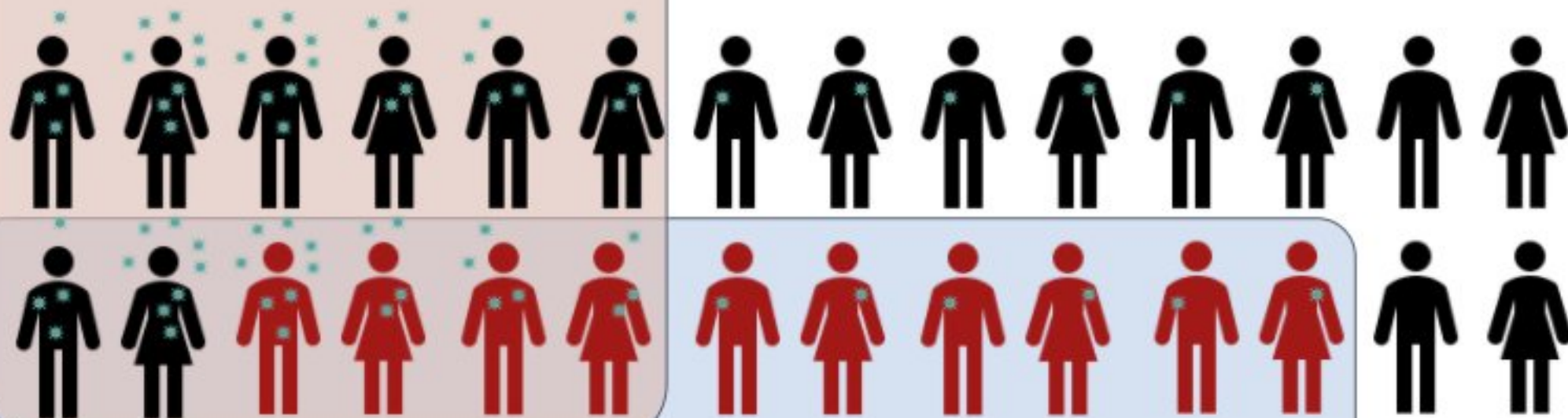
To treat the disease, find these people

April 15, 2020

Carlos Cordon-Cardo, MD, Ph.D.
Mount Sinai Laboratory
Center for Clinical Laboratories
Annenberg Building Floor 15 Room 15-60A
1468 Madison Ave
New York, NY 10029

Device:	COVID-19 ELISA IgG Antibody Test
Company:	Mount Sinai Laboratory
Indication:	Qualitative detection of human IgG antibodies in serum and plasma specimens collected from individuals suspected of prior infection with the virus that causes COVID-19 by their healthcare provider. Emergency use of this test is limited to the Mount Sinai Laboratory (MSL), Center for Clinical Laboratories, a division of the Department of Pathology, Molecular, and Cell-Based Medicine, New York, NY, that is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a to perform high complexity tests ("authorized laboratory").

Dear Dr. Carlos Cordon-Cardo:

This letter is in response to your[1] request that the Food and Drug Administration (FDA) issue an Emergency Use Authorization (EUA) for emergency use of your product,[2] pursuant to Section 564 of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. §360bbb-3).

On February 4, 2020, pursuant to Section 564(b)(1)(C) of the Act, the Secretary of the Department of Health and Human Services (HHS) determined that there is a public health emergency that has a significant potential to affect national security or the health and security of United States citizens living abroad, and that involves the virus that causes COVID-19. Pursuant to Section 564 of the Act, and on the basis of such determination, the Secretary of HHS then declared that circumstances exist justifying the authorization of emergency use of in

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[1] For ease of reference, this letter will use the term "you" and related terms to refer to the Mount Sinai Laboratory.
[2] For ease of reference, this letter will use the term "your product" to refer to the COVID-19 ELISA IgG Antibody Test used for the indication identified above.

vitro diagnostics for detection and/or diagnosis of the virus that causes COVID-19 subject to the terms of any authorization issued under Section 564(a) of the Act.[3]

Having concluded that the criteria for issuance of this authorization under Section 564(c) of the Act are met, I am authorizing the emergency use of your product, described in the Scope of Authorization of this letter (Section II), subject to the terms of this authorization.

I. Criteria for Issuance of Authorization

I have concluded that the emergency use of your product meets the criteria for issuance of an authorization under Section 564(c) of the Act, because I have concluded that:

1. The SARS-CoV-2 can cause a serious or life-threatening disease or condition, including severe respiratory illness, to humans infected by this virus;

2. Based on the totality of scientific evidence available to FDA, it is reasonable to believe that your product may be effective in diagnosing prior infection with the virus that causes COVID-19, and that the known and potential benefits of your product when used for diagnosing prior infection with the virus that causes COVID-19, outweigh the known and potential risks of your product; and,

3. There is no adequate, approved, and available alternative to the emergency use of your product.[4]

II. Scope of Authorization

I have concluded, pursuant to Section 564(d)(1) of the Act, that the scope of this authorization is limited to the indication above.

Authorized Product Details

Your product is a qualitative test for the detection of IgG antibodies against SARS-CoV-2 in serum and plasma specimens collected from individuals suspected of prior infection with the virus that causes COVID-19 by their healthcare provider. Results are for the detection of SARS-CoV-2 IgG antibodies that are generated as part of the human immune response to the virus. IgG antibodies to SARS-CoV-2 generally become detectable 10 – 14 days after infection although may be detected earlier. Positive results for IgG antibodies can be indicative of an immune response to acute or previous infection; however, testing for IgG antibodies should not be used for the diagnosis of acute infection.

Your product has been developed for the qualitative detection of human SARS-CoV-2 antibody in serum and plasma via serial Enzyme-Linked ImmunoSorbent Assays (ELISA). The initial ELISA test assay controls and patient samples diluted 1:50 with PBS are added to a Thermo

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[3] U.S. Department of Health and Human Services, *Determination of a Public Health Emergency and Declaration that Circumstances Exist Justifying Authorizations Pursuant to Section 564(b) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3*. 85 FR 7316 (February 7, 2020).
[4] No other criteria of issuance have been prescribed by regulation under Section 564(c)(4) of the Act.

Scientific Immulon 96 well microtiter plate that was coated with SARS-CoV-2 recombinant Receptor Binding Domain protein (RBD). The coated RBD protein combines with patient's SARS-CoV-2 IgG antibodies and the plate is then washed. Secondary anti-human IgG HRP labeled antibody is then added, washed, and substrate for HRP is added. When the value of color is greater than the cut-off value (OD490 = 0.15) the specimen is a presumptive positive. Presumptive positive specimens (OD490 > 0.15) are then confirmed by an ELISA using dilutions of specimen in a Thermo Scientific Immulon 96 well microtiter plate coated with full length SARS-CoV-2 Spike protein. A confirmed positive has a result greater than the cut-off value (OD490 = 0.15) at a titer of 1:80 or above, while a negative is OD490 ≤ 0.15 at a titer of 1:80.

Your product also includes external positive and negative controls, or other authorized controls, to be run as outlined in the Instructions for Use, including:

- Positive Controls are prepared using remnant pooled serum that was tested positive for SARS-CoV-2 antibodies by direct ELISA. The positive control absorbance at 490 nm must be > 0.15.
- Negative Controls are prepared using remnant pooled serum that was tested negative for SARS-CoV-2 antibodies by direct ELISA. The negative control absorbance at 490 nm must be less than 0.15.

Your product also requires the use of additional authorized materials and authorized ancillary reagents that are not included with your product and are described in the Instructions for Use.

The above described product, when labeled consistently with the authorized procedures submitted as part of the EUA request, and as described in the EUA summary (available at https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations), which may be revised in consultation with, and with concurrence of, the Division of Microbiology Devices (DMD)/Office of Health Technology 7 Office of In Vitro Diagnostics and Radiological Health (OHT7-OIR)/Office of Product Evaluation and Quality (OPEQ)/Center for Devices and Radiological Health (CDRH), is authorized to be distributed to and used by the authorized laboratory under this EUA, despite the fact that it does not meet certain requirements otherwise required by applicable federal law.

Your product is authorized to be accompanied by the following product-specific information pertaining to the emergency use, which is required to be made available to healthcare providers and patients:

- Fact Sheet for Healthcare Providers: COVID-19 ELISA IgG Antibody Test
- Fact Sheet for Patients: COVID-19 ELISA IgG Antibody Test

I have concluded, pursuant to Section 564(d)(2) of the Act, that it is reasonable to believe that the known and potential benefits of your authorized product, when used for diagnosing prior infection with the virus that causes COVID-19 and used consistently with the Scope of Authorization of this letter (Section II), outweigh the known and potential risks of your product.

I have concluded, pursuant to Section 564(d)(3) of the Act, based on the totality of scientific

evidence available to FDA, that it is reasonable to believe that your product may be effective for the indication above, when used consistently with the Scope of Authorization of this letter (Section II), pursuant to Section 564(c)(2)(A) of the Act.

FDA has reviewed the scientific information available to FDA, including the information supporting the conclusions described in Section I above, and concludes that your product (as described in the Scope of Authorization of this letter (Section II)) meets the criteria set forth in Section 564(c) of the Act concerning safety and potential effectiveness.

The emergency use of your product under this EUA must be consistent with, and may not exceed, the terms of this letter, including the Scope of Authorization (Section II) and the Conditions of Authorization (Section IV). Subject to the terms of this EUA and under the circumstances set forth in the Secretary of HHS's determination under Section 564(b)(1)(C) described above and the Secretary of HHS's corresponding declaration under Section 564(b)(1), your product is authorized for the indication above.

This EUA will cease to be effective when the HHS declaration that circumstances exist to justify the EUA is terminated under Section 564(b)(2) of the Act or when the EUA is revoked under Section 564(g) of the Act.

III. Waiver of Certain Requirements

I am waiving the following requirements for your product during the duration of this EUA:

- Current good manufacturing practice requirements, including the quality system requirements under 21 CFR Part 820 with respect to the design, manufacture, packaging, labeling, storage, and distribution of your product.

IV. Conditions of Authorization

Pursuant to Section 564(e) of the Act, I am establishing the following conditions on this authorization:

Mount Sinai Laboratory (MSL), Center for Clinical Laboratories

A. Your product must comply with the following labeling requirements under FDA regulations: the intended use statement (21 CFR 809.10(a)(2), (b)(2)); adequate directions for use (21 U.S.C. 352(f)), (21 CFR 809.10(b)(5), (7), and (8)); any appropriate limitations on the use of the device including information required under 21 CFR 809.10(a)(4); and any available information regarding performance of the device, including requirements under 21 CFR 809.10(b)(12).

B. You will inform relevant public health authorities of this EUA, including the terms and conditions herein, and any updates made to your product, authorized labeling and

authorized Fact Sheets.

C. You will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

D. You will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

E. You will make available on your website(s) the Fact Sheet for Healthcare Providers and the Fact Sheet for Patients.

F. You are authorized to make available additional information relating to the emergency use of your product that is consistent with, and does not exceed, the terms of this letter of authorization.

G. You will use your authorized test as outlined in the authorized test procedures submitted as part of the EUA request. Deviations from the authorized test procedures, will be made in consultation with, and require concurrence of, DMD/OHT7-OIR/OPEQ/CDRH.

H. You will collect information on the performance of your product. You will report to FDA to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) any suspected occurrence of false positive and false negative results and significant deviations from the established performance characteristics of the product of which you become aware.

I. You may request changes to the Scope of Authorization (Section II in this letter) of your product. Such requests will be made in consultation with DMD/OHT7-OIR/OPEQ/CDRH, and require concurrence of, Office of Counterterrorism and Emerging Threats (OCET)/Office of the Chief Scientist (OCS)/Office of the Commissioner (OC) and DMD/OHT7-OIR/OPEQ/CDRH.

J. You may request changes to the authorized labeling. Such requests will be made in consultation with, and require concurrence of, DMD/OHT7-OIR/OPEQ/CDRH.

K. You may request changes to the authorized Fact Sheets. Such requests will be made in consultation with, and require concurrence of, DMD/OHT7-OIR/OPEQ/CDRH.

L. You may request the addition of other instruments for use with your product. Such requests will be made in consultation with, and require concurrence of, DMD/OHT7-OIR/OPEQ/CDRH.

M. You may request the addition of other ancillary methods for use with your product. Such requests will be made in consultation with, and require concurrence of, DMD/OHT7-OIR/OPEQ/CDRH.

N. You may request the addition of other specimen types for use with your product. Such requests will be made in consultation with, and require concurrence of, DMD/OHT7-OIR/OPEQ/CDRH.

O. You may request the addition and/or substitution of control materials for use with your product. Such requests will be made in consultation with, and require concurrence of, DMD/OHT7-OIR/OPEQ/CDRH.

P. You may request substitution for or changes to the authorized materials used in the detection process of human antibodies against SARS-CoV-2. Such requests will be made in consultation with, and require concurrence of, DMD/OHT7-OIR/OPEQ/CDRH.

Q. You will evaluate the performance and assess traceability[5] of your product with any FDA-recommended reference material(s) or established panel(s) of characterized clinical specimens. After submission to FDA and DMD/OHT7-OIR/CDRH's review of and concurrence with the data, you will update your labeling to reflect the additional testing. Such labeling updates will be made in consultation with, and require concurrence of, DMD/OHT7-OIR/OPEQ/CDRH.

R. You will track adverse events, including any occurrence of false results and report to FDA under 21 CFR Part 803.

S. All laboratory personnel using your product must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in, and be familiar with, the interpretation of results of the product.

T. You will maintain records of test usage and ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

Conditions Related to Advertising and Promotion

U. All advertising and promotional descriptive printed matter relating to the use of your product shall be consistent with the Fact Sheets and authorized labeling, as well as the terms set forth in this EUA and the applicable requirements set forth in the Act and FDA regulations.

V. All advertising and promotional descriptive printed matter relating to the use of your

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[5] Traceability refers to tracing analytical sensitivity/reactivity back to an FDA-recommended reference material.

product shall clearly and conspicuously state that:

- This test has not been FDA cleared or approved;

- This test has been authorized by FDA under an EUA for use by authorized laboratories;

- This test has been authorized only for the detection of IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens; and,

- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

No advertising or promotional descriptive printed matter relating to the use of your product may represent or suggest that this test is safe or effective for the detection of SARS-CoV-2.

The emergency use of your product as described in this letter of authorization must comply with the conditions and all other terms of this authorization.

V. Duration of Authorization

This EUA will be effective until the declaration that circumstances exist justifying the authorization of the emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 is terminated under Section 564(b)(2) of the Act or the EUA is revoked under Section 564(g) of the Act.

Sincerely,

_____
RADM Denise M. Hinton
Chief Scientist
Food and Drug Administration

Enclosures

nCoV Spike Protein Receptor Binding Domain Shares High Amino Acid Identity With a Coronavirus Recovered from a Pangolin Viral Metagenomic Dataset

An outbreak of respiratory illness caused by a novel coronavirus (nCoV-2019, NC_045512.2) first identified in Wuhan China has resulted in over seven thousand confirmed cases. So far, the nCoV-2019 has been reported to share 96% sequence identity to the RaTG13 genome (EPI_ISL_402131) – Figure 1A. However, the S1 Receptor Binding Domain (RBD) of the nCoV-2019 genome was noticeably divergent between the two at amino acid residues 350 to 550. We aimed to identity coronaviruses related to nCoV-2019 in viral metagenomics datasets available in the public domain. In a recently published dataset describing viral diversity in Malayan pangolins (doi:10.3390/v11110979, PRJNA573298) we used VirMAP (doi.org/10.1038/s41467-018-05658-8) to reconstruct a coronavirus genome (approximately 84% complete from samples SRR10168377 and SRR10168378) that shared 97% amino acid identity across the same RBD segment – Figure 1B. This result indicates a potential recombination event for nCoV-2019.

NC_045512.2
https://www.ncbi.nlm.nih.gov/nuccore/NC_045512.2

EPI_ISL_402131
https://gisaid.org/CoV2020

Malayan Pangolins Paper
https://doi:10.3390/v11110979

Malayan Pangolins Dataset
https://www.ncbi.nlm.nih.gov/bioproject/573298

VirMAP paper
https://doi.org/10.1038/s41467-018-05658-8

VirMAP – Pangolin Coronavirus fasta:

Figure 1

A. nCoV-2019 vs. RaTG13

surface glycoprotein [Wuhan seafood market pneumonia virus]

Sequence ID: YP_009724390.1 Length: **1273** Number of Matches: **6**

See 5 more title(s) ⌄

Range 1: 350 to 550 GenPept   Graphics   ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
384 bits(986)	1e-126	Compositional matrix adjust.	181/201(90%)	190/201(94%)	0/201(0%)	+2

```
Query  22592  VYAWNRKRISNCVADYSVLYNSTSFSTFKCYGVSPTKLNDLCFTNVYADSFVITGDEVRQ  22771
              VYAWNRKRISNCVADYSVLYNS SFSTFKCYGVSPTKLNDLCFTNVYADSFVI GDEVRQ
Sbjct  350   VYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQ  409

Query  22772  IAPGQTGKIADYNYKLPDDFTGCVIAWNSKHIDAKEGGNFNYLYRLFRKANLKPFERDIS  22951
              IAPGQTGKIADYNYKLPDDFTGCVIAWNS ++D+K GGN+NYLYRLFRK+NLKPFERDIS
Sbjct  410   IAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS  469

Query  22952  TEIYQAGSKPCNGQTGLNCYYPLYRYGFYPTDGVGHQPYRVVVLSFELLNAPATVCGPKK  23131
              TEIYQAGS PCNG  G NCY+PL   YGF PT+GVG+QPYRVVVLSFELL+APATVCGPKK
Sbjct  470   TEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKK  529

Query  23132  STNLVKNKCVNFNFNGLTGTG   23194
              STNLVKNKCVNFNFNGLTGTG
Sbjct  530   STNLVKNKCVNFNFNGLTGTG   550
```

B. nCoV-2019 vs. Pangolin CoV

surface glycoprotein [Wuhan seafood market pneumonia virus]

Sequence ID: YP_009724390.1 Length: **1273** Number of Matches: **5**

See 5 more title(s) ⌄

Range 1: 350 to 545 GenPept   Graphics   ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
404 bits(1039)	1e-133	Compositional matrix adjust.	190/196(97%)	193/196(98%)	0/196(0%)	+1

```
Query  22321  VYAWNRKRISNCVADYSVLYNSTSFSTFKCYGVSPTKLNDLCFTNVYADSFVVRGDEVRQ  22500
              VYAWNRKRISNCVADYSVLYNS SFSTFKCYGVSPTKLNDLCFTNVYADSFV+RGDEVRQ
Sbjct  350   VYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQ  409

Query  22501  IAPGQTGRIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS  22680
              IAPGQTG+IADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS
Sbjct  410   IAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS  469

Query  22681  TEIYQAGSTPCNGVEGFNCYFPLQSYGFHPTNGVGYQPYRVVVLSFELLXAPATVCGPKQ  22860
              TEIYQAGSTPCNGVEGFNCYFPLQSYGF PTNGVGYQPYRVVVLSFELL APATVCGPK+
Sbjct  470   TEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKK  529

Query  22861  STNLVKNKCVNFNFNG   22908
              STNLVKNKCVNFNFNG
Sbjct  530   STNLVKNKCVNFNFNG   545
```

Figure 1

A. nCoV-2019 vs. RaTG13

surface glycoprotein [Wuhan seafood market pneumonia virus]

Sequence ID: **YP_009724390.1**   Length: **1273**   Number of Matches: **6**

See 5 more title(s) ⌄

Range 1: 350 to 550 GenPept   Graphics   ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
384 bits(986)	1e-126	Compositional matrix adjust.	181/201(90%)	190/201(94%)	0/201(0%)	+2

```
Query  22592  VYAWNRKRISNCVADYSVLYNSTSFSTFKCYGVSPTKLNDLCFTNVYADSFVITGDEVRQ  22771
              VYAWNRKRISNCVADYSVLYNS SFSTFKCYGVSPTKLNDLCFTNVYADSFVI GDEVRQ
Sbjct  350    VYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQ  409

Query  22772  IAPGQTGKIADYNYKLPDDFTGCVIAWNSKHIDAKEGGNFNYLYRLFRKANLKPFERDIS  22951
              IAPGQTGKIADYNYKLPDDFTGCVIAWNS ++D+K GGN+NYLYRLFRK+NLKPFERDIS
Sbjct  410    IAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS  469

Query  22952  TEIYQAGSKPCNGQTGLNCYYPLYRYGFYPTDGVGHQPYRVVVLSFELLNAPATVCGPKK  23131
              TEIYQAGS PCNG  G NCY+PL  YGF PT+GVG+QPYRVVVLSFELL+APATVCGPKK
Sbjct  470    TEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKK  529

Query  23132  STNLVKNKCVNFNFNGLTGTG     23194
              STNLVKNKCVNFNFNGLTGTG
Sbjct  530    STNLVKNKCVNFNFNGLTGTG     550
```

B. nCoV-2019 vs. Pangolin CoV

surface glycoprotein [Wuhan seafood market pneumonia virus]

Sequence ID: **YP_009724390.1**   Length: **1273**   Number of Matches: **5**

See 5 more title(s) ⌄

Range 1: 350 to 545 GenPept   Graphics   ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
404 bits(1039)	1e-133	Compositional matrix adjust.	190/196(97%)	193/196(98%)	0/196(0%)	+1

```
Query  22321  VYAWNRKRISNCVADYSVLYNSTSFSTFKCYGVSPTKLNDLCFTNVYADSFVVRGDEVRQ  22500
              VYAWNRKRISNCVADYSVLYNS SFSTFKCYGVSPTKLNDLCFTNVYADSFV+RGDEVRQ
Sbjct  350    VYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQ  409

Query  22501  IAPGQTGRIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS  22680
              IAPGQTG+IADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS
Sbjct  410    IAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS  469

Query  22681  TEIYQAGSTPCNGVEGFNCYFPLQSYGFHPTNGVGYQPYRVVVLSFELLXAPATVCGPKQ  22860
              TEIYQAGSTPCNGVEGFNCYFPLQSYGF PTNGVGYQPYRVVVLSFELL APATVCGPK+
Sbjct  470    TEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKK  529

Query  22861  STNLVKNKCVNFNFNG     22908
              STNLVKNKCVNFNFNG
Sbjct  530    STNLVKNKCVNFNFNG     545
```

RESEARCH ARTICLE
MICROBIOLOGY

On the origin and continuing evolution of SARS-CoV-2

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3. Center for Biosafety Mega-Science, Chinese Academy of Sciences, China
4. University of Chinese Academy of Sciences, China
5. School of Life Sciences, Shanghai University, China
6. NHC Key Laboratory of Systems Biology of Pathogens, Institute of Pathogen Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing
7. These authors contributed equally to this work.

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ABSTRACT

The SARS-CoV-2 epidemic started in late December 2019 in Wuhan, China, and has since impacted a large portion of China and raised major global concern. Herein, we investigated the extent of molecular divergence between SARS-CoV-2 and other related coronaviruses. Although we found only 4% variability in genomic nucleotides between SARS-CoV-2 and a bat SARS-related coronavirus (SARSr-CoV; RaTG13), the difference at neutral sites was 17%, suggesting the divergence between the two viruses is much larger than previously estimated. Our results suggest that the development of new variations in functional sites in the receptor-binding domain (RBD) of the spike seen in SARS-CoV-2 and viruses from pangolin SARSr-CoVs are likely caused by mutations and natural selection besides recombination. Population genetic analyses of 103 SARS-CoV-2 genomes indicated that these viruses evolved into two major types (designated L and S), that are well defined by two different SNPs that show nearly complete linkage across the viral strains sequenced to date. Although the L type (~70%) is more prevalent than the S type (~30%), the S type was found to be the ancestral version. Whereas the L type was more prevalent in the early stages of the outbreak in Wuhan, the frequency of the L type decreased after early January 2020. Human intervention may have placed more severe selective pressure on the L type, which might be more aggressive and spread more quickly. On the other hand, the S type, which is evolutionarily older and less aggressive, might have increased in relative frequency due to relatively weaker selective pressure. These findings strongly support an urgent need for further immediate, comprehensive studies that combine genomic data, epidemiological data, and chart records of the clinical symptoms of patients with coronavirus disease 2019 (COVID-19).

Keywords: SARS-CoV-2, virus, molecular evolution, population genetics

INTRODUCTION

The coronavirus disease 2019 (COVID-19) epidemic started in late December 2019 in Wuhan, the capital of Central China's Hubei Province. Since then, it has rapidly spread across China and in other countries, raising major global concerns. The etiological agent is a novel coronavirus, SARS-CoV-2, named for the similarity of its symptoms to those induced by the severe acute respiratory syndrome. As of February 28, 2020, 78,959 cases of SARS-CoV-2 infection have been confirmed in China, with 2,791 deaths. Worryingly, there have also been more than 3,664 confirmed cases outside of China in 46 countries and areas (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/), raising significant doubts about the likelihood of successful containment. Further, the genomic sequences of SARS-CoV-2 viruses isolated from a number of patients share sequence identity higher than 99.9%, suggesting a very recent host shift into humans [1-3].

Coronaviruses are naturally hosted and evolutionarily shaped by bats [4, 5]. Indeed, it has been postulated that most of the coronaviruses in humans are derived from the bat reservoir [6, 7]. Unsurprisingly, several teams have recently confirmed the genetic similarity between SARS-CoV-2 and a bat betacoronavirus of the sub-genus *Sarbecovirus* [8-13]. The whole-genome sequence identity of the novel virus has 96.2% similarity to a bat SARS-related coronavirus (SARSr-CoV; RaTG13) collected in Yunnan province, China [2, 14], but is not very similar to the genomes of SARS-CoV (about 79%) or MERS-CoV (about 50%) [1, 15]. It has also been confirmed that the SARS-CoV-2 uses the same receptor, the angiotensin converting enzyme II (ACE2), as the SARS-CoV [11]. Although the specific route of transmission from natural reservoirs to humans remains unclear [5, 13], several studies have shown that pangolins may have provided a partial *spike* gene to SARS-CoV-2; the critical functional sites in the spike protein of SAR-CoV-2 are nearly identical to one identified in a virus isolated from a pangolin [16-18].

Despite these recent discoveries, several fundamental issues related to the evolutionary patterns and driving forces behind this outbreak of SARS-CoV-2 remain unexplored [19]. Herein, we investigated the extent of molecular divergence between SARS-CoV-2 and other related coronaviruses and carried out population genetic analyses of 103 sequenced genomes of SARS-CoV-2. This work provides new insights into the factors driving the evolution of SARS-CoV-2 and its pattern of spread through the human population.

RESULTS

Molecular phylogeny and divergence between SARS-CoV-2 and related coronaviruses.
For each annotated ORF in the reference genome of SARS-CoV-2 (NC_045512), we extracted the orthologous sequences in human SARS-CoV, four bat

SARS-related coronaviruses (SARSr-CoV: RaTG13, ZXC21, ZC45, and BM48-31), one Pangolin SARSr-CoV from Guangdong (GD) [17], and six Pangolin SARSr-CoV genomes from Guangxi (GX) [18] (Table S1). We aligned the coding sequences (CDSs) based on the protein alignments (see Materials and Methods). Most ORFs annotated from SARS-CoV-2 were found to be conserved in other viruses, except for *ORF8* and *ORF10* (Table 1). The protein sequence of SARS-CoV-2 *ORF8* shared very low similarity with sequences in SARS-CoV and BM48-31, and *ORF10* had a premature stop codon in both SARS-CoV and BM48-31 (Fig. S1). A one-base deletion caused a frame-shift mutation in *ORF10* of ZXC21 (Fig. S1).

To investigate the phylogenetic relationships between these viruses at the genomic scale, we concatenated coding regions (CDSs) of the nine conserved ORFs (*orf1ab, E, M, N, S, ORF3a, ORF6, ORF7a,* and *ORF7b*) and reconstructed the phylogenetic tree using the synonymous sites (Fig. 1A). We also used CODEML in the PAML [20] to infer the ancestral sequence of each node and calculated the dN (nonsynonymous substitutions per nonsynonymous site), dS (synonymous substitutions per synonymous site), and dN/dS (ω) values for each branch (Fig. 1A). In parallel, we also calculated the pairwise dN, dS, and ω values between SARS-CoV-2 and another virus (Table 1).

The genome-wide phylogenetic tree indicated that SARS-CoV-2 was closest to RaTG13, followed by GD Pangolin SARSr-CoV, then by GX Pangolin SARSr-CoVs, then by ZC45 and ZXC21, then by human SARS-CoV, and finally by BM48-31(Fig. 1A). Notably, we found that the nucleotide divergence at synonymous sites between SARS-CoV-2 and other viruses was much higher than previously anticipated. For example, although the overall genomic nucleotides overall differ ~4% between SARS-CoV-2 and RaTG13, the genomic average dS was 0.17, which means the divergence at the neutral sites is 17% between these two viruses (Table 1). This is because the nonsynonymous sites are usually under stronger negative selection than synonymous sites, and calculating sequence differences without separating these two classes of sites may underestimate the extent of molecular divergence by several folds.

Notably, the dS value varied considerably across genes in SARS-CoV-2 and the other viruses analyzed. In particular, the *spike* gene (*S*) consistently exhibited larger dS values than other genes (Table 1). This pattern became clear when we calculated the dS value for each branch in Fig. 1A for the *spike* gene versus the concatenated sequences of the remaining genes (Fig. S2). In each branch, the dS of *spike* was 2.22 ± 1.35 (mean $\pm$ SD) times as large as that of the other genes. This extremely elevated dS value of *spike* could be caused either by a high mutation rate or by natural selection that favors synonymous substitutions. Synonymous

substitutions may serve as another layer of genetic regulation, guiding the efficiency of mRNA translation by changing codon usage [21]. If positive selection is the driving force for the higher synonymous substation rate seen in *spike*, we expect the frequency of optimal codons (FOP) of *spike* to be different from that of other genes. However, our codon usage bias analysis (Table S2) suggests the FOP of *spike* was only slightly higher than that of the genomic average (0.717 versus 0.698, see Materials and Methods). Thus, we believe that the elevated synonymous substitution rate measured in *spike* is more likely caused by higher mutational rates; however, the underlying molecular mechanism remains unclear.

Both SARS-CoV and SARS-CoV-2 bind to ACE2 through the RBD of spike protein in order to initiate membrane fusion and enter human cells [1, 2, 22-26]. Five out of the six critical amino acid (AA) residues in RBD were different between SARS-CoV-2 and SARS-CoV (Fig. 1B), and a 3D structural analysis indicated that the spike of SARS-CoV-2 has a higher binding affinity to ACE2 than SARS-CoV [23]. Intriguingly, these same six critical AAs are identical between GD Pangolin-CoV and SARS-CoV-2 [16]. In contrast, although the genomes of SARS-CoV-2 and RaTG13 are more similar overall, only one out of the six functional sites are identical between the two viruses (Fig. 1B). It has been proposed that the SARS-CoV-2 RBD region of the spike protein might have resulted from recent recombination events in pangolins [16-18]. Although several ancient recombination events have been described in *spike* [27, 28], it also seems likely that the identical functional sites in SARS-CoV-2 and GD Pangolin-CoV may actually the result of coincidental convergent evolution [18].

If the functional AA residues in the SARS-CoV-2 RBD region were acquired from GD Pangolin-CoV in a very recent recombination event, we would expect the nucleotide sequences of this region to be nearly identical between the two viruses. However, for the CDS sequences that span five critical AA sites in the SARS-CoV-2 spike (ranging from codon 484 to 507, covering five adjacent functional sites: F486, Q493, S494, N501, and Y505; Fig. S3), we estimated dS = 0.411, dN = 0.019, and ω= 0.046 between SARS-CoV-2 and GD Pangolin-CoV. By assuming the synonymous substitution rate (*u*) of 1.67-4.67 x 10^{-3}/site/year, as estimated in SARS-CoV [29], the recombination/introgression, if it occurred at all, would be estimated to happen approximately 19.8-55.4 years ago. Here, the formula $t = dS/(u \times 2 \times 2.22)$ was used to calculate divergence time; note that the increased mutational rate of *spike* was considered for this calculation. Thus, it seems very unlikely that SARS-CoV-2 originated from the GD Pangolin-CoV due to a very recent recombination event. Alternatively, it seems more likely that a high mutation rate in *spike*, coupled with strong natural selection, has shaped the identical functional AA residues between these two viruses, as proposed previously [18]. Although these sites are maintained in SARS-CoV-2 and GD

Pangolin-CoV, mutations may have changed the residues in the RaTG13 lineage after it diverged from SARS-CoV-2 (the blue arrow in Fig. 1A). In summary, it seems that the shared identity of critical AA sites between SARS-CoV-2 and GD Pangolin-CoV might be due to random mutations coupled with natural selection, and not necessarily recombination.

Selective constraints and positive selection during the evolution of SARS-CoV-2 and related coronaviruses

The genome-wide ω value between SARS-CoV-2 and other viruses ranged from 0.044 to 0.124 (Table 1), indicative of strong negative selection on the nonsynonymous sites. In other words, 87.6% to 95.6% of the nonsynonymous mutations were removed by negative selection during viral evolution. To determine the extent of positive selection, we concatenated the CDS sequences of 9 conserved ORFs in all the viruses in Fig. 1A and fitted the M7 (beta: neutral and negative selection) and M8 (beta + ω>1:neutral, negative selection, and positive selection) model using CODEML (Materials and Methods). The M8 model (lnL = -104,813.732, np =18) was a significantly better fit than the M7 (lnL = -105,063.284, np = 16) model ($P < 10^{-10}$), suggesting that some AA substitutions were favored by positive Darwinian selection (but not necessarily in the SARS-CoV-2 lineage).Under the M8 model, 98.48% (p_0) of the nonsynonymous substitutions were estimated under neutral evolution or purifying selection (0⩽ω⩽1), and 1.52% (p_1) of the nonsynonymous substitutions were under positive selection (ω = 1.50). A Bayes Empirical Bayes (BEB) analysis suggested that 10 AA sites showed strong signals of positive selection, and, interestingly, three of those were located in the RBD of spike, including at one critical site (Fig. 1C and Fig. S4). Thus, although these coronaviruses were generally under very strong negative selection, positive selection was also responsible for the evolution of protein sequences. The putatively positively-selected sites might serve as candidates for further functional studies.

Mutations in 103 SARS-CoV-2 genomes

We downloaded 103 publicly available SARS-CoV-2 genomes, aligned the sequences, and identified the genetic variants. For ease of visualization, we marked each virus strain based on the location and date the virus was isolated with the format of "Location_Date" throughout this study (see Table S1 for details; Each ID did not contain information of the patient's race or ethnicity). Although SARS-CoV-2 is an RNA virus, for simplicity, we presented our results based on DNA sequencing results throughout this study (*i.e.*, the nucleotide T (thymine) means U (uracil) in SARS-CoV-2). For each variant, the ancestral state was inferred based on the genome and CDS alignments of SARS-CoV-2 (NC_045512), RaTG13, and GD Pangolin-CoV (Materials and Methods). In total, we identified mutations in 149 sites across the 103 sequenced strains. Ancestral states for 43 synonymous, 83 non-synonymous, and two stop-gain mutations were unambiguously inferred. The frequency spectra of synonymous and nonsynonymous mutations are shown in Fig. 2.

Most derived mutations were singletons (67.4% (29/43) of synonymous mutations and 84.3% (70/83) of nonsynonymous mutations), indicating either a recent origin [30] or population growth [31]. In general, the derived alleles of synonymous mutations were significantly skewed towards higher frequencies than those of nonsynonymous ones ($P < 0.01$, Wilcoxon rank-sum test; Fig. 2), suggesting the nonsynonymous mutations tended to be selected against. However, 16.3% (7 out of 43) synonymous mutations, and one nonsynonymous (ORF8 (L84S, 28,144)) mutation had a derived frequency of $\geq 70\%$ across the SARS-CoV2 strains. The nonsynonymous mutations that had derived alleles in at least two SARS-CoV-2 strains affected six proteins: orf1ab (A117T, I1607V, L3606F, I6075T), S (H49Y, V367F), ORF3a (G251V), ORF7a (P34S), ORF8 (V62L, S84L), and N (S194L, S202N, P344S).

Two major types of SARS-CoV-2 are defined by two SNPs that show complete linkage

To detect the possible recombination among SARS-CoV2 viruses, we used Haploview [32] to analyze and visualize the patterns of linkage disequilibrium (LD) between variants with minor alleles in at least two SARS-CoV-2 strains (Fig. 3A). Since most mutations were at very low frequencies, it is not surprising that many pairs had a very low r^2 or LOD value (Fig. 3B-C). Consistent with another recent report [31], we did not find evidence of recombination between the SARS-CoV2 strains.

However, we found that SNPs at location 8,782 (*orf1ab*: T8517C, synonymous) and 28,144 (*ORF8*: C251T, S84L) showed significant linkage, with an r^2 value of 0.954 (Fig. 3B, red) and a LOD value of 50.13 (Fig. 3C, red). Among the 103 SARS-CoV-2 virus strains, 101 of them exhibited complete linkage between the two SNPs: 72 strains exhibited a "CT" haplotype (defined as "L" type because T28,144 is in the codon of Leucine) and 29 strains exhibited a "TC" haplotype (defined as "S" type because C28,144 is in the codon of Serine) at these two sites. Thus, we categorized the SARS-CoV-2 viruses into two major types, with L being the major type (~70%) and S being the minor type (~30%).

The evolutionary history of L and S types of SARS-CoV-2

Although we defined the L and S types based on two tightly linked SNPs, strikingly, the separation between the L (blue) and S (red) types was maintained when we reconstructed the haplotype networks using all the SNPs in the SARS-CoV-2 genomes (Fig. 4A; the number of mutations between two neighboring haplotypes was inferred parsimoniously). This analysis further supports the idea that the two linked SNPs at sites 8,782 and 28,144 adequately define the L and S types of SARS-CoV-2.

To determine whether L or S type is ancestral, we examined the genomic alignments of SARS-CoV-2 and other highly related viruses. Strikingly, nucleotides of the S type at sites 8,782 and 28,144 were identical to the orthologous sites in the most closely related viruses (Fig. 4B). Remarkably, both sites were highly conserved in other viruses as well. Hence, although the L type (~70%) was more prevalent than the S type (~30%) in the SARS-CoV-2 viruses we examined, the S type is actually the ancestral version of SARS-CoV-2.

To further examine the relationship among the strains in the L and S types, we reconstructed a phylogenetic tree of all the 103 SARS-CoV-2 viruses based on their whole-genome sequences. Our phylogenetic tree also clearly shows the separation of the two types (Fig. 5). Viruses of the L type (blue) first clustered together, and likewise, viruses of the S type (red) were also more closely related to each other. Therefore, our whole-genome comparisons further confirm the separation of the L and S types.

Thus far, we found that, although the L type is derived from the S type, L (~70%) is more prevalent than S (~30%) among the sequenced SARS-CoV-2 genomes we examined. This pattern suggests that L has a higher transmission rate than the S type. Furthermore, our mutational load analysis indicated that the L type had accumulated a significantly higher number of derived mutations than S type ($P < 0.0001$, Wilcoxon rank-sum test; Fig. S5). We propose that, although the L type newly evolved from the ancient S type, it transmits faster or replicates faster in human populations, causing it to accumulate more mutations than the S type. Thus, our results suggest the L might be more aggressive than the S type due to the potentially higher transmission and/or replication rates.

To test whether the two types of SARS-CoV-2 had differences in temporal and spatial distributions, we stratified the viruses based on the locations and dates they were isolated (Table S1). Among the 27 viruses isolated from Wuhan, 26 (96.3%) were L type, and only 1 (3.7%) was S type. However, among the other 73 viruses isolated outside Wuhan, 45 (61.6%) were L type, and 28 (38.4%) were S type. This comparison suggests that the L type is significantly more prevalent in Wuhan than in other places ($P = 0.0004$, Fisher's exact test, Fig. 6 and Table S3). All of the 26 samples isolated before January 7, 2020, were from Wuhan, and among the 74 samples collected from January 7, 2020, only one was from Wuhan, 33 were from other places in China, and 40 were from patients outside China. Thus, it is not surprising that the L type was significantly more prevalent before January 7, 2020 (96.2%, 25 L and 1 S) than after January 7, 2020 (62.2%, 46 L and 28 S) ($P = 0.0008$, Fisher's exact test, Fig. 6 and Table S3).

If the L type is more aggressive than the S type, why did the relative frequency of the L type decrease compared to the S type in other places after the initial breakout in Wuhan? One possible explanation is that, since January 2020, the Chinese central and local governments have taken rapid and comprehensive prevention and control measures. These human intervention efforts might have caused severe selective pressure against the L type, which might be more aggressive and spread more quickly. The S type, on the other hand, might have experienced weaker selective pressure by human intervention, leading to an increase in its relative abundance among the SARS-CoV-2 viruses. Thus, we hypothesized that the two types of SARS-CoV-2 viruses might have experienced different selective pressures due to different epidemiological features. Of note, the above analyses were based on very patchy SARS-CoV-2 genomes that were collected from different locations and time points. More comprehensive genomic data is required for further testing of our hypothesis.

Heteroplasmy of SARS-CoV-2 viruses in patients

It is currently unclear how the L type specifically evolved from the S type during the development of SARS-CoV-2. However, we found that the sequence of viruses isolated from one patient that lived in the United States on January 21 (USA_2020/01/21.a, GISAID ID: EPI_ISL_404253) had the genotype Y (C or T) at both positions 8,782 and 28,144, differing from the general trend of having either C or T. Although novel mutations could lead to this result, the most parsimonious explanation is that this patient may have been infected by both the L and S types (Fig. 7A). The sample of USA_2020/01/21.a was collected from a 63-year-old female patient living in Chicago (from GISAID). Based on the report from the United States Centers for Disease Control and Prevention (https://www.cdc.gov/media/releases/2020/p0124-second-travel-coronavirus.html), we inferred this patient returned to the United States from Wuhan on January 13, 2020. However, whether the co-existence of L and S types in this patient was due to multiple-time infections during her visit to Wuhan is currently unclear. Notably, the viruses identified from a patient in Australia on January 28, 2020 (Australia_2020/01/28.a, GISAID ID: EPI_ISL_407894) had multiple degenerate nucleotides. This sample was collected from a 44-year-old male patient in Gold Cost, Australia (from GISAID). Based on the report from the Courier Mail (January 30, 2020), we inferred this patient had the history of traveling from Wuhan to the Gold Coast before the diagnosis of infection. As shown in Fig. 7B, we inferred this patient might have been infected by at least two different strains of SARS-CoV-2 (Fig. 7B).

To further investigate the heteroplasmy of SARS-CoV-2 viruses in patients, we searched 12 deep-sequencing libraries of SARS-CoV-2 genomes that were deposited in the Sequence Read Archive (SRA) (Table S4, Materials and Methods). We found 17 genomic sites that showed evidence of heteroplasmy of SARS-CoV-2 virus in five patients, but we did not find

any other instances of the co-existence of L and S types in any patient (Table 2). These findings evince the developing complexity of the evolution of SARS-CoV-2 infections. Further studies investigating how the different alleles of SARS-CoV-2 viruses compete with each other will be of significant value.

DISCUSSION

In this study, we investigated the patterns of molecular divergence between SARS-CoV-2 and other related coronaviruses. Although the genomic analyses suggested that SARS-CoV-2 was closest to RaTG13, their difference at neutral sites was much higher than previously realized. Our results provide novel insights into tracing the intermediate natural host of SARS-CoV-2. With population genetic analyses of 103 genomes of SARS-CoV-2, we found that SARS-CoV-2 viruses evolved into two major types (L and S types), and the two types were well defined by just two SNPs that show nearly complete linkage across SARS-CoV-2 strains. Although the L type (~70%) was more prevalent than the S type (~30%) in the SARS-CoV-2 viruses we examined, our evolutionary analyses suggested the S type was most likely the more ancient version of SARS-CoV-2. Our results also support the idea that the L type is more aggressive than the S type.

Since nonsynonymous sites are usually under stronger negative selection than synonymous sites, calculating sequence differences without separating these two classes of sites could lead to a potentially significant underestimate of the degree of molecular divergence. For example, although the overall nucleotides only differed by ~4% between SARS-CoV-2 and RaTG13, the genomic average dS value, which is usually a neutral proxy, was 0.17 between these two viruses (Table 1). Of note, the genome-wide dS value is 0.012 between humans and chimpanzees [33], and 0.08 between humans and rhesus macaques [34]. Thus, the neutral molecular divergence between SARS-CoV-2 and RaTG13 is 14 times larger than that between humans and chimpanzees, and twice as large as that between humans and macaques. The genomic average dS value between SARS-CoV-2 and GD Pangolin-CoV is 0.475, which is comparable to that between humans and mice (0.5) [35], and the dS value between SARS-CoV-2 and GX Pangolin-Cov is even larger (0.722). The scale of these measures suggests that we should perhaps consider the difference in the neutral evolving site rather than the difference in all nucleotide sequences when tracing the origin and natural intermediate host of SARS-CoV-2.

Our analyses of molecular evolution and population genetics suggested that some amino acid changes might be favored by natural selection during the evolution of SARS-CoV-2 and other related viruses. However, negative selection appears to be the predominant force acting on these viruses. Interestingly, the virus isolated from one patient in Shenzhen on January 13,

2020 (SZ_2020/01/13.a, GISAID ID: EPI_ISL_406592) had C at both positions 8,782 and 28,144 in the genome, belonging to neither L nor S type (Fig. 4A and 5). Notably, this strain had one stop-gain mutation in *orf1ab* and had accumulated 20 silent and 5 nonsynonymous mutations after diverging from the ancestor haplotype (Fig. 4A). Thus, it is possible that functional constraints on the genomic sequence were weakened after the disruption of *orf1ab* in this strain. Notably, on viruses isolated from a patient living in South Korean (Skorea_2020/01.a, GISAID: EPI_ISL_411929), acquired six nonsynonymous mutations that were different from the most recent common ancestor of SARS-CoV-2: orf1ab (M902I and T6891M), S (S221W), ORF3a (W128L and G251V), and E (L37H). If these changes are not due to sequencing errors, it would be interesting to test whether and how these mutations affect the transmission and pathogenesis of SARS-CoV-2.

In this work, we propose that SARS-CoV-2 can be divided into two major types (L and S types): the S type is ancestral, and the L type evolved from S type. Intriguingly, the S and L types can be clearly defined by just two tightly linked SNPs at positions 8,782 (*orf1ab*: T8517C, synonymous) and 28,144 (*ORF8*: C251T, S84L). However, it is currently unclear whether L type evolved from the S type in humans or in the intermediate hosts. It is also unclear whether the L type is more virulent than the S type. *orf1ab*, which encodes replicase/transcriptase, is required for viral genome replication and might also be important for viral pathogenesis [36]. Although the T8517C mutation in *orf1ab* does not change the protein sequence (it changes the codon AGT (Ser) to AGC (Ser)), we hypothesized this mutation might affect *orf1ab* translation since AGT is preferred while AGC is unpreferred (Table S2). ORF8 promotes the expression of ATF6, the ER unfolded protein response factor, in human cells [37]. Thus, it will be interesting to investigate the function of the S84L AA change in ORF8, as well as the combinatory effect of these two mutations in SARS-CoV-2 pathogenesis.

In summary, our analyses of 103 sequenced SARS-CoV-2 genomes suggest that the L type is more aggressive than the S type and that human interference may have shifted the relative abundance of L and S type soon after the SARS-CoV-2 outbreak. As previously noted [19], the data examined in this study are still very limited, and follow-up analyses of a larger set of data are needed to have a better understanding of the evolution and epidemiology of SARS-CoV-2. There is a strong need for further immediate, comprehensive studies that combine genomic data, epidemiological data, and chart records of the clinical symptoms of patients with SARS-CoV-2.

MATERIALS AND METHODS

Molecular evolution of SARS-CoV-2 and other related viruses

The set of 103 complete genome sequences were downloaded from GISAID (Global Initiative on Sharing All Influenza Data; https://www.gisaid.org/) with acknowledgment, GenBank (https://www.ncbi.nlm.nih.gov/genbank), and NMDC (http://nmdc.cn/#/nCoV). Sequences and annotations of the reference genome of SARS-CoV-2 (NC_045512) and other related viruses were downloaded from GenBank or GISAID (Table S1). The genomic sequences of SARS-CoV-2 were aligned using MUSCLE v3.8.31 [38].

The annotated CDSs of other viruses were downloaded from GenBank. To avoid missing annotations in other viruses, we also annotated the ORFs using CDSs annotated in SARS-CoV-2 using Exonerate (--model protein2genome:bestfit --score 5 -g y) [39]. The protein sequences of SARS-CoV-2 and other related viruses were aligned with MUSCLE v3.8.31 [38], and the codon alignments were made based on the protein alignment with RevTrans [40]. The codon alignments of the conserved ORFs were further concatenated for down-stream evolutionary analysis. The phylogenetic tree was constructed by the neighbor-joining method in MEGA-X [41] using the parameters of Kimura 2-parameter model, and only the third positions of codons were considered. YN00 from PAML v4.9a [20] was used to calculate the pairwise divergence between SARS-CoV-2 and other viruses for each individual gene or for the concatenated sequences. The free-ratio model in CODEML in the PAML [20] package was used to calculate the dN, dS, and ω values for each branch.

Positively selected amino acids

Positive selection was detected using EasyCodeML [42], a recently published wrapper of CODEML [20]. The M7 and M8 models were compared. In the M7 model, ω follows a beta distribution such that $0 \leqslant \omega \leqslant 1$, and in the M8 model, a proportion p_0 of sites have ω drawn from the beta distribution, and the remaining sites with proportion p_1 are positively selected and have $\omega_1 > 1$. The LRTs between M7 and M8 models were conducted by comparing twice the difference in log-likelihood values ($2 \ln \Delta l$) against a χ^2-distribution (df=2). The positively selected sites were identified with the Bayes Empirical Bayes (BEB) score larger than 0.95.

Haplotype network

DnaSP v6.12.03 [43] was used to generate multi-sequence aligned haplotype data, and PopART v1.7 [44] was used to draw haplotype networks based on the haplotypes generated by DnaSP. RAxML v8.2.12 [45] was used to build the maximum likelihood phylogenetic tree of 103 aligned SARS-CoV-2 genomes with theparameters "-p 1234 -m GTRCAT".

SNP calling process

We downloaded 12 SARS-CoV-2 metagenomic sequencing libraries (Table S2), and mapped the NGS reads to the reference genome of SARS-CoV-2 (NC_045512) using BWA (0.7.17-r1188) [46] with the default parameters. SNP calling was done using bcftools mpileup (bcftools 1.9) [47].

Codon usage bias analysis

We calculated the RSCU (Relative Synonymous Codon Usage) value of each codon in the SARS-CoV-2 reference genome (NC_045512). The RSCU value for each codon was the observed frequency of this codon divided by its expected frequency under equal usage among the amino acid [48]. The codons with RSCU > 1 were defined as preferred codons, and those with RSCU < 1 were defined as unpreferred codons. The FOP (frequency of optimal codons) value of each gene was calculated as the number of preferred codons divided by the total number of preferred and unpreferred codons.

Conflict of interest

The authors declare that they have no conflicts of interest.

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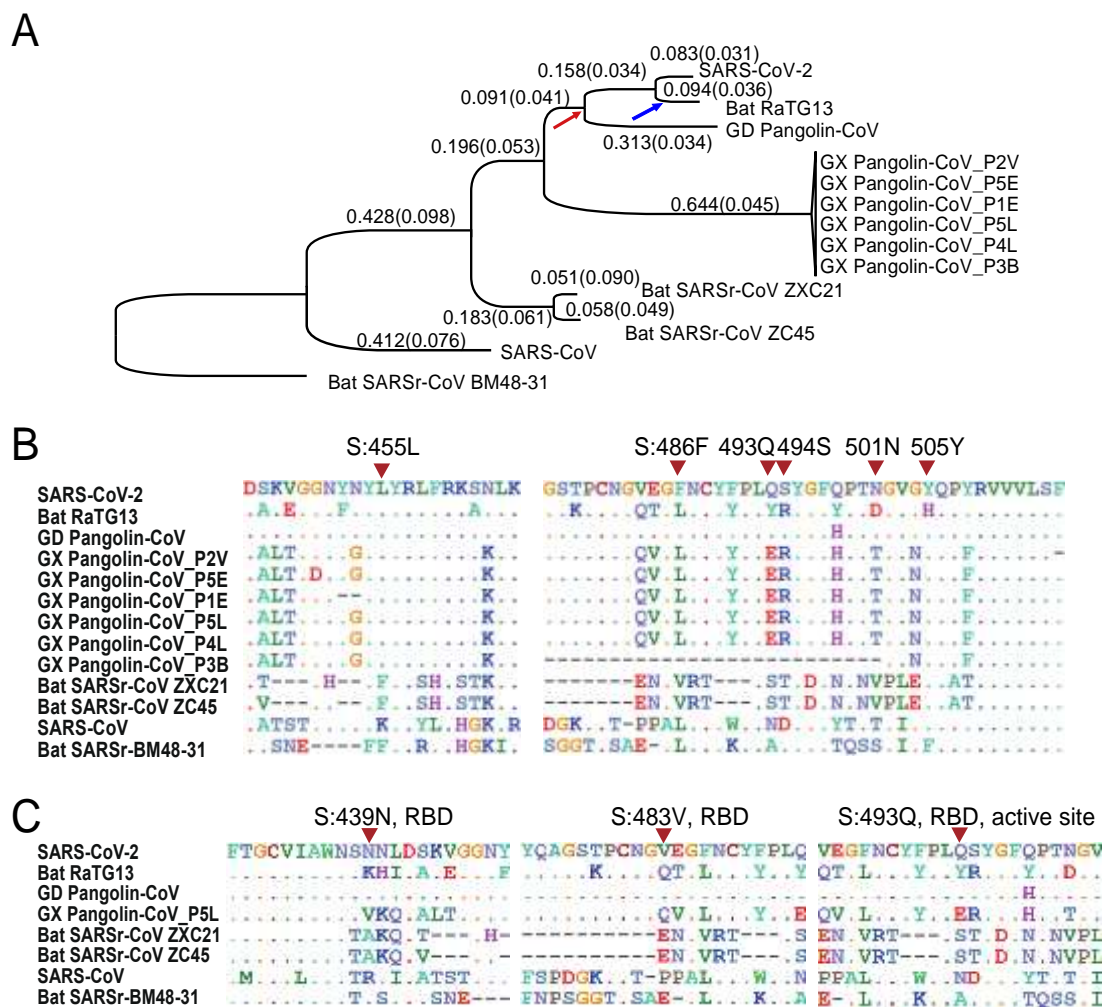
Figure 1. Molecular divergence and selective pressures during the evolution of SARS-CoV-2 and related viruses.
A. The phylogenetic tree of SARS-CoV-2 and the related Coronaviruses. The branch length (dS) is presented, and the dN/dS (ω) value is given in the parenthesis. The phylogenetic tree was reconstructed with the synonymous sites in the concatenated CDSs of nine conserved ORFs (*orf1ab, E, M, N, S, ORF3a, ORF6, ORF7a* and *ORF7b*).
B. Conservation of 6 critical amino acid residues in the spike (S) protein. The critical active sites are Y442, L472, N479, D480, T487, and Y491 in SARS-CoV, and they correspond to L455, F486, Q493, S494, N501, and Y505 in SARS-CoV-2 (marked with inverted triangles), respectively.
C. Three candidate positively selected sites (marked with inverted triangles) in the receptor-binding domain (RBD) of spike protein (S:439N, S:483V and S:493Q) and the surrounding 10 amino acids.
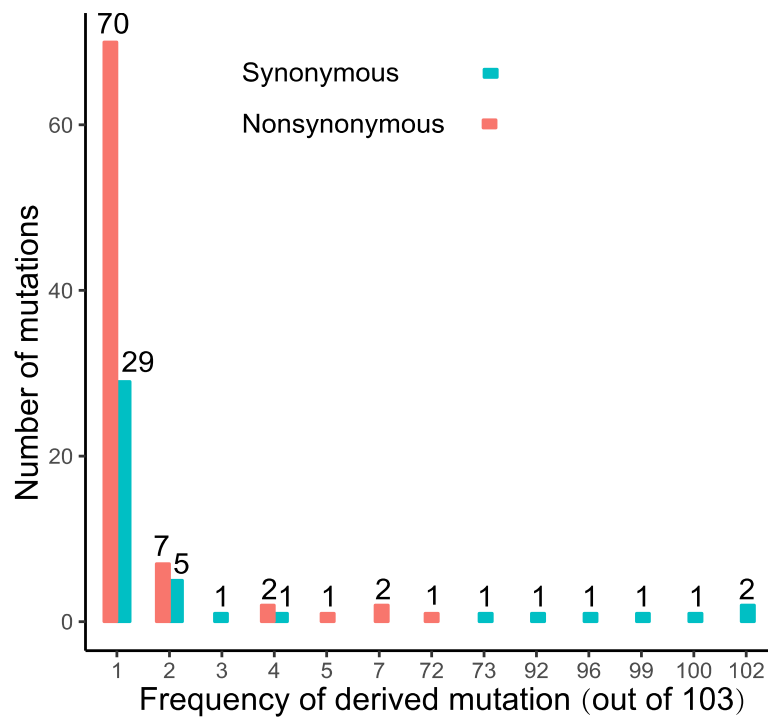
Figure 2. The frequency spectra of derived mutations in 103 SARS-CoV-2 viruses. Note the derived alleles of synonymous mutations are skewed towards higher frequencies than those of nonsynonymous mutations.
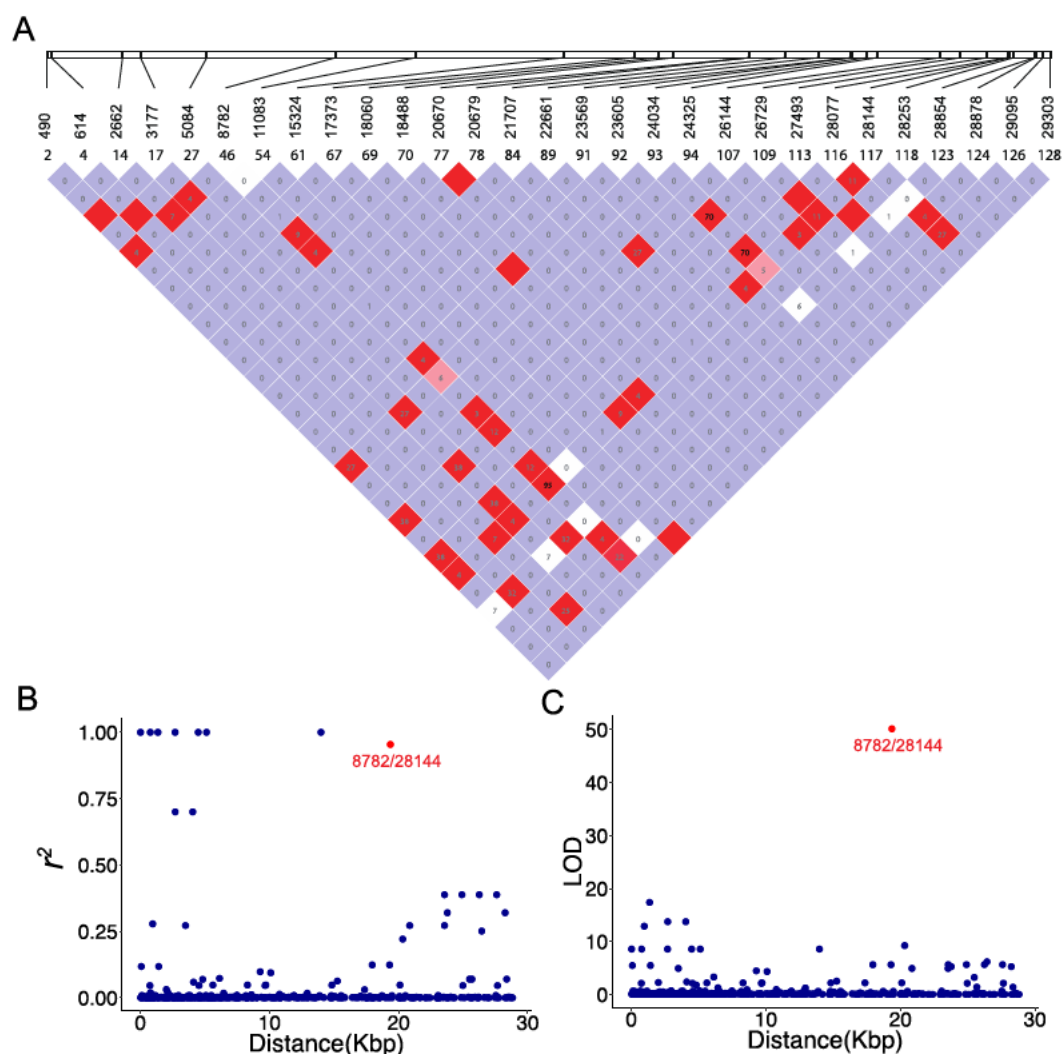
Figure 3. Linkage disequilibrium between SNPs in the SARS-CoV-2 viruses.
A. LD plot of any two SNP pairs among the 29 sites that have minor alleles in at least two strains. The number near slashes at the top of the image shows the coordinate of sites in the genome. Color in the square is given by standard (D'/LOD), and the number in square is r^2 value.
B. The r^2 of each pair of SNPs (y-axis) against the genomic distance between that pair (x-axis).
C. The LOD of each pair of SNPs (y-axis) against the genomic distance between that pair (x-axis).
Note that in both **B** and **C**, the red point represents the LD between SNPs at 8,782 and 28,144.
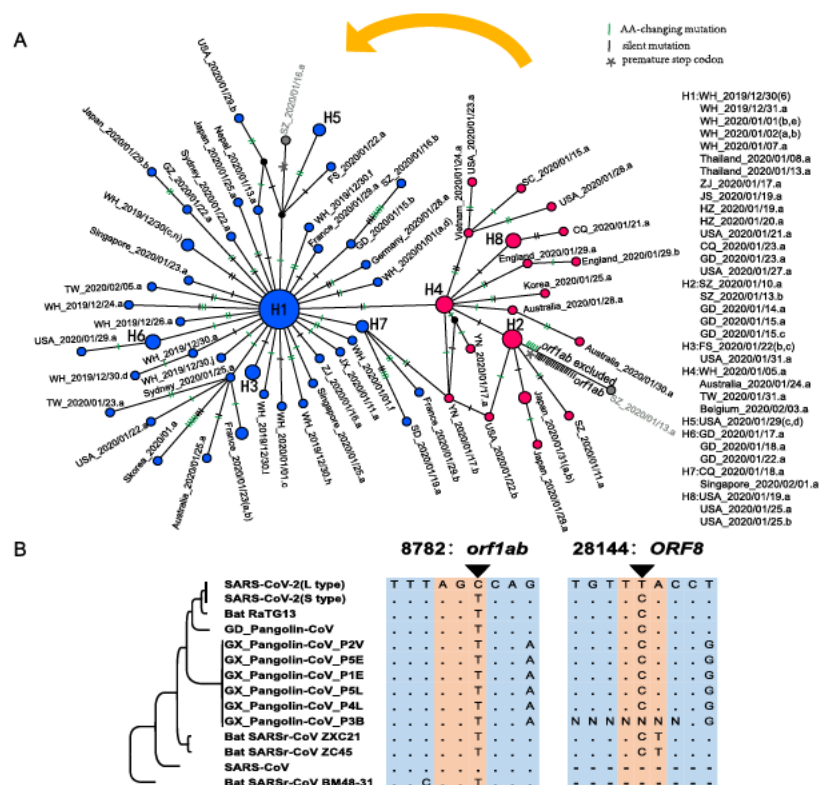
Figure 4. Haplotype analysis of SARS-CoV-2 viruses.

A. The haplotype networks of SARS-CoV-2 viruses. Blue represents the L type, and red is the S type. The orange arrow indicates that the L type evolved from the S type. Note that in this study, we marked each sample with a unique ID that starting with the geological location, followed by the date the virus was isolated (see Table S1 for details). Each ID did not contain information of the patient's race or ethnicity. ZJ, Zhejiang; YN, Yunnan; WH, Wuhan; USA, United States of America; TW, Taiwan; SZ, Shenzhen; SD, Shandong; SC, Sichuan; JX, Jiangxi; JS, Jiangsu; HZ, Hangzhou; GZ, Guangzhou; GD, Guangdong; FS, Foshan; CQ, Chongqing.

B. Evolution of the L and S types of SARS-CoV-2 viruses. Genome sequence alignments with the seven most closely related viruses indicated that the S type was most likely the ancient version of SARS-CoV-2. ".", The nucleotide sequence is identical; "-", gap.

Figure 5. The unrooted phylogenetic tree of the 103 SARS-CoV-2 genomes. The ID of each sample is the same as in Fig. 4A. Note WH_2019/12/31.a represents the reference genome (NC_045512). Note SZ_2020/01/13.a had C at both positions 8,782 and 28,144 in the genome, belonging to neither L nor S type.
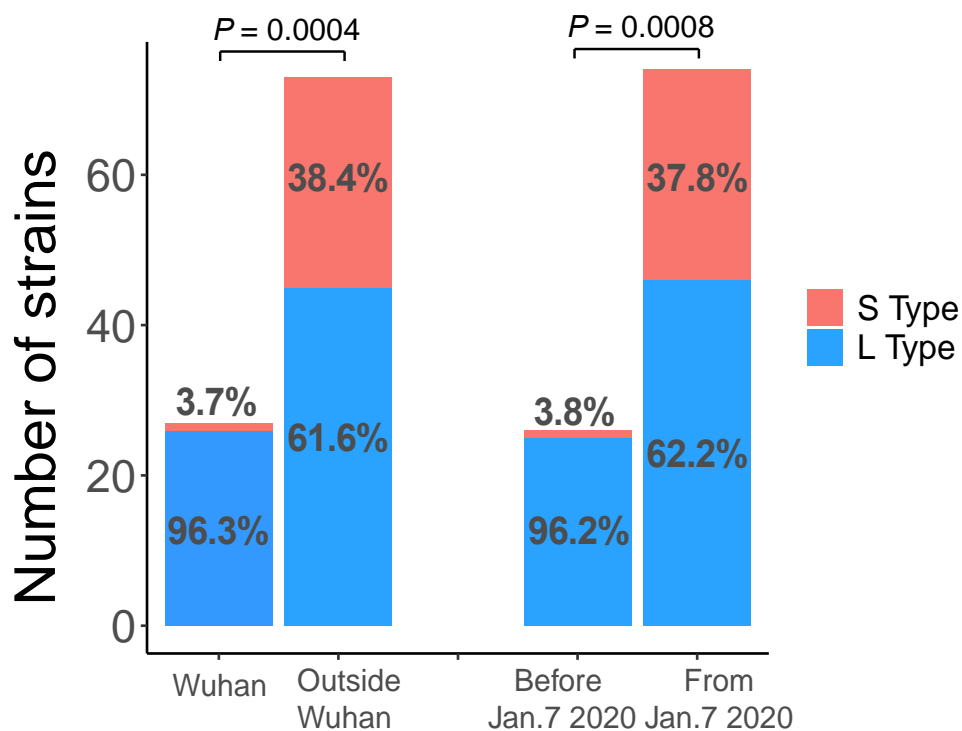
Figure 6. The two types of SARS-CoV-2 showed differences in temporal and spatial distributions.
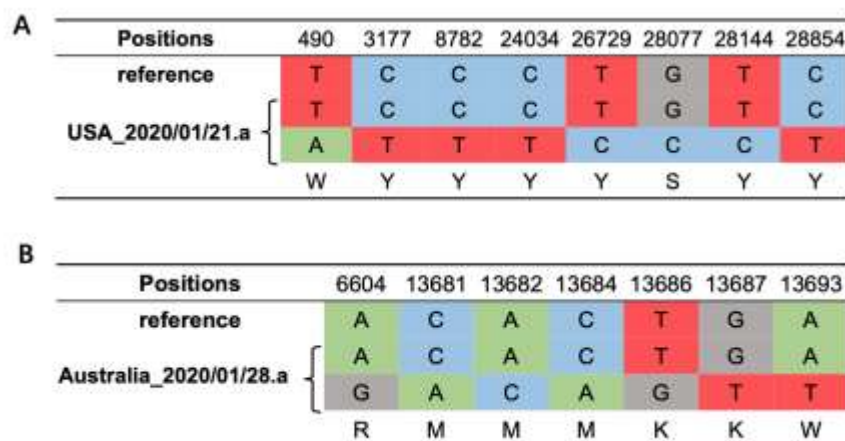
Figure 7. The heteroplasmy of SARS-CoV-2 viruses in human patients.
A. The viruses isolated from a patient that lived in the United States (USA_2020/01/21.a, GISAID ID: EPI_ISL_404253) had the genotype Y (C or T) at both 8,782 and 28,144. The most likely explanation is that this patient was infected by both the L and S types. Note the reference is L type.
B. The viruses Australia_2020/01/28.a (GISAID ID:EPI_ISL_407894) identified from a patient in Australia had multiple degenerated nucleotides, and the best explanation is that this patient was infected by at least two different strains of SARS-CoV-2 viruses.

Table 1 The molecular divergence between SARS-CoV-2 and related viruses

Gene	Aligned Length (nt)	RaTG13	GD Pangolin-CoV	GX Pangolin-CoV	SARSr-CoV ZC45	SARS-CoV	SARSr-CoV BM48-31
Genomic Average	28734	0.008/0.17 (0.044)	0.026/0.475 (0.054)	0.055/0.722 (0.076)	0.044/0.549 (0.081)	0.113/0.926 (0.122)	0 143/1.15 (0.124)
ORF10	114	0.011/0 (NA)	0.011/0 (NA)	0.072/0.044 (1.637)	0.011/0 (NA)	-	-
ORF3a	825	0.009/0.157 (0.06)	0.019/0.291 (0.065)	0.066/0.518 (0.128)	0.052/0.508 (0.102)	0.188/0.918 (0.205)	0 271/0.923 (0.294)
ORF6	183	0/0.098 (0)	0.014/0.217 (0.062)	0.038/0.491 (0.077)	0.027/0.173 (0.158)	0.191/0.913 (0.209)	0 393/1.512 (0.26)
ORF7a	363	0.011/0.177 (0.061)	0.018/0.275 (0.066)	0.073/0.477 (0.153)	0.066/0.351 (0.188)	0.088/0.697 (0.126)	0 337/1.14 (0.296)
ORF7b	129	0.01/0 (NA)	0.02/0.455 (0.043)	0.17/0.436 (0.39)	0.029/0.181 (0.162)	0.155/0.401 (0.387)	0.264/NA (NA)
ORF8	363	0.021/0.07 (0.303)	0.032/0.303 (0.105)	0.099/1.015 (0.098)	0.03/0.603 (0.05)	-	-
E	225	0/0.018 (0)	0/0.037 (0)	0.006/0.096 (0.063)	0/0.056 (0)	0.027/0.166 (0.164)	0.043/0.352 (0.121)
M	666	0.004/0.186 (0.021)	0.014/0.298 (0.046)	0.025/0.372 (0.067)	0.016/0.283 (0.055)	0.07/0.576 (0.121)	0 109/1.292 (0.085)
N	1257	0.005/0.131 (0.039)	0.011/0.144 (0.076)	0.04/0.304 (0.132)	0.036/0.333 (0.108)	0.059/0.381 (0.155)	0 102/1.197 (0.085)
orf1a	13215	0.009/0.167 (0.054)	0.026/0.488 (0.053)	0.073/0.811 (0.09)	0.026/0.405 (0.063)	0.148/1.141 (0.129)	0 174/1.199 (0.145)
orf1ab	21288	0.007/0.152 (0.044)	0.019/0.495 (0.039)	0.055/0.776 (0.071)	0.031/0.527 (0.058)	0.105/0.962 (0.109)	0 125/1.108 (0.113)
S (*spike*)	3819	0.014/0.321 (0.043)	0.075/0.69 (0.108)	0.06/0.86 (0.07)	0.138/1.063 (0.13)	0.172/1.265 (0.136)	0 217/1.518 (0.143)

For each gene, the dN and dS values between SARS-CoV-2 and another virus are given, and the dN/dS (ω) ratio is given in the parenthesis.

Table 2. The heteroplasmy of SARS-CoV-2 viruses in human patients

Accession number	Genomic position	Ref allele	Alt allele	Ref reads	Alt reads	Location_date	GISAID ID
SRR10903401	1821	G	A	52	5	WH_2020/01/02.a	EPI_ISL_406716
SRR10903401	19164	C	T	40	12	WH_2020/01/02.a	EPI_ISL_406716
SRR10903401	24323	A	C	102	67	WH_2020/01/02.a	EPI_ISL_406716
SRR10903401	26314	G	A	15	2	WH_2020/01/02.a	EPI_ISL_406716
SRR10903401	26590	T	C	10	2	WH_2020/01/02.a	EPI_ISL_406716
SRR10903402	11563	C	T	164	26	WH_2020/01/02.b	EPI_ISL_406717
SRR11092057	9064	TTAT	TT	13	2	WH_2019/12/30.e	EPI_ISL_402124
SRR11092057	17825	C	T	19	5	WH_2019/12/30.e	EPI_ISL_402124
SRR11092059	4795	C	T	10	4	WH_2019/12/30.h	EPI_ISL_402130
SRR11092059	6360	A	G	39	5	WH_2019/12/30.h	EPI_ISL_402130
SRR11092059	7042	G	A	5	3	WH_2019/12/30.h	EPI_ISL_402130
SRR11092059	12153	C	T	15	13	WH_2019/12/30.h	EPI_ISL_402130
SRR11092059	15921	G	T	19	2	WH_2019/12/30.h	EPI_ISL_402130
SRR11092059	16474	A	G	11	2	WH_2019/12/30.h	EPI_ISL_402130
SRR11092059	20344	C	T	19	2	WH_2019/12/30.h	EPI_ISL_402130
SRR11092062	565	T	C	64	23	WH_2019/12/30.e	EPI_ISL_402124
SRR11092062	17825	C	T	141	34	WH_2019/12/30.e	EPI_ISL_402124
SRR11092063	29441	C	A	6	2	WH_2019/12/30.d	EPI_ISL_402127

Addendum to

On the origin and continuing evolution of SARS-CoV-2　(DOI: 10.1093/nsr/nwaa036)

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In our recent publication (https://doi.org/10.1093/nsr/nwaa036), we showed that among circulating SARS-CoV-2 (with 103 genomes analyzed) two different viral genomes co-exist. We identified them as lineages L and S. The concerned amino acid we used to define the L and S lineages is located in ORF8 (open reading frame 8), which plays a yet undefined role in the viral life cycle. Based on the finding that "L" lineage has a higher frequency than lineage S, we described the L lineage as aggressive. We now recognize that within the context of our study the term "aggressive" is misleading and should be replaced by a more precise term "a higher frequency".　In short, while we have shown that the two lineages naturally co-exist, we provided no evidence supporting any epidemiological conclusion regarding the virulence or pathogenicity of SARS-CoV-2. By saying so, corrections will be made in the print version of this paper to avoid being misleading.

中文

在我们最近发表的文章（ https://doi.org/10.1093/nsr/nwaa036 ）中，分析结果显示，103个 SARS-CoV-2 病毒基因组存在两种不同的谱系；分别称之为 "L"和"S"谱系。我们用来定义 L 和 S 谱系的氨基酸位点位于 ORF8(开放阅读框 8)基因，这个基因还没有发现具有任何已知的重要功能。基于" L"谱系的频率高于谱系 S 的发现，我们将 L 谱系描述为"aggressive"（具有侵略性）。我们现在认识到，在本研究阐述的内容中，"侵略性"一词

会具有误导性，应该用更精确的术语"更高的频率"代替。 简而言之，尽管我们已经发现这两个谱系自然并存，但我们没有提供任何证据支持关于 SARS-CoV-2 毒力或致病性的任何流行病学结论。 因此，我们将在本文的印刷版本中进行更正，以避免产生误导。

Prolonged presence of SARS-CoV-2 viral RNA in faecal samples

We present severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR results of all respiratory and faecal samples from patients with coronavirus disease 2019 (COVID-19) at the Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, China, throughout the course of their illness and obligated quarantine period. Real-time RT-PCR was used to detect COVID-19 following the recommended protocol (appendix p 1). Patients with suspected SARS-CoV-2 were confirmed after two sequential positive respiratory tract sample results. Respiratory and faecal samples were collected every 1–2 days (depending on the availability of faecal samples) until two sequential negative results were obtained. We reviewed patients' demographic information, underlying diseases, clinical indices, and treatments from their official medical records. The study was approved by the Medical Ethical Committee of The Fifth Affiliated Hospital of Sun Yat-sen University (approval number K162-1) and informed consent was obtained from participants. Notably, patients who met discharge criteria were allowed to stay in hospital for extended observation and health care.

Between Jan 16 and March 15, 2020, we enrolled 98 patients. Both respiratory and faecal samples were collected from 74 (76%) patients. Faecal samples from 33 (45%) of 74 patients were negative for SARS CoV-2 RNA, while their respiratory swabs remained positive for a mean of 15·4 days (SD 6·7) from first symptom onset. Of the 41 (55%) of 74 patients with faecal samples that were positive for SARS-CoV-2 RNA, respiratory samples remained positive for SARS-CoV-2 RNA for a mean of 16·7 days (SD 6·7) and faecal samples remained positive for a mean of 27·9 days (10·7) after first symptom onset (ie, for a mean of 11·2 days [9·2] longer than for respiratory samples). The full disease course of the 41 patients with faecal samples that were positive for SARS-CoV-2 RNA is shown in the figure. Notably, patient 1 had positive faecal samples for 33 days continuously after the respiratory samples became negative, and patient 4 tested positive for SARS-CoV-2 RNA in their faecal sample for 47 days after first symptom onset (appendix pp 4–5).

A summary of clinical symptoms and medical treatments is shown in the appendix (pp 2–3, 6–8). The presence of gastrointestinal symptoms was not associated with faecal sample viral RNA positivity (p=0·45); disease severity was not associated with extended duration of faecal sample viral RNA positivity (p=0·60); however, antiviral treatment was positively associated with the presence of viral RNA in faecal samples (p=0·025; appendix pp 2–3). These associations should be interpreted with caution because of the possibility of confounding. Additionally, the Ct values of all three targeted genes (RdRp, N, E) in the first faecal sample that was positive for viral RNA were negatively associated with the duration of faecal viral RNA positivity (RdRp gene r =−0·34; N gene

Figure: Timeline of results from throat swabs and faecal samples through the course of disease for 41 patients with SARS-CoV-2 RNA positive faecal samples, January to March, 2020

$r = -0.02$; and E gene $r = -0.16$), whereas the correlation of the Ct values with duration of faecal sample positivity was only significant for $RdRp$ (p=0.033; N gene p=0.91; E gene p=0.33).

Our data suggest the possibility of extended duration of viral shedding in faeces, for nearly 5 weeks after the patients' respiratory samples tested negative for SARS-CoV-2 RNA. Although knowledge about the viability of SARS-CoV-2 is limited,[1] the virus could remain viable in the environment for days, which could lead to faecal–oral transmission, as seen with severe acute respiratory virus CoV and Middle East respiratory syndrome CoV.[2] Therefore, routine stool sample testing with real-time RT-PCR is highly recommended after the clearance of viral RNA in a patient's respiratory samples. Strict precautions to prevent transmission should be taken for patients who are in hospital or self-quarantined if their faecal samples test positive.

As with any new infectious disease, case definition evolves rapidly as knowledge of the disease accrues. Our data suggest that faecal sample positivity for SARS-CoV-2 RNA normally lags behind that of respiratory tract samples; therefore, we do not suggest the addition of testing of faecal samples to the existing diagnostic procedures for COVID-19. However, the decision on when to discontinue precautions to prevent transmission in patients who have recovered from COVID-19 is crucial for management of medical resources. We would suggest the addition of faecal testing for SARS-CoV-2.[3] Presently, the decision to discharge a patient is made if they show no relevant symptoms and at least two sequential negative results by real-time RT-PCR of sputum or respiratory tract samples collected more than 24 h apart. Here, we observed that for over half of patients, their faecal samples remained positive for SARS-CoV-2 RNA for a mean of 11·2 days after respiratory tract samples became negative for SARS-CoV-2 RNA, implying that the virus is actively replicating in the patient's gastrointestinal tract and that faecal–oral transmission could occur after viral clearance in the respiratory tract.

Determining whether a virus is viable using nucleic acid detection is difficult; further research using fresh stool samples at later timepoints in patients with extended duration of faecal sample positivity is required to define transmission potential. Additionally, we found patients normally had no or very mild symptoms after respiratory tract sample results became negative (data not shown); however, asymptomatic transmission has been reported.[4] No cases of transmission via the faecal–oral route have yet been reported for SARS-CoV-2, which might suggest that infection via this route is unlikely in quarantine facilities, in hospital, or while under self-isolation. However, potential faecal–oral transmission might pose an increased risk in contained living premises such as hostels, dormitories, trains, buses, and cruise ships.

Respiratory transmission is still the primary route for SARS-CoV-2 and evidence is not yet sufficient to develop practical measures for the group of patients with negative respiratory tract sample results but positive faecal samples. Further research into the viability and infectivity of SARS-CoV-2 in faeces is required.

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Study Title: Evaluation of a novel point-of-care diagnostic for SARS-CoV-2

MD Anderson Informed Consent

Protocol Number:
Approval Date:
Expiration Date:

Researchers at MD Anderson: Kathleen Schmeler, MD; Keyur Patel, MD, PhD; Ignacio Wistuba, MD

_____   _____
Participant's Name   Medical Record Number

This consent and authorization form explains why the research study is being done and what your role will be if you choose to take part. You may choose not to take part in this study.

STUDY SUMMARY
The goal of this clinical research study is to evaluate a new test for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes the disease called coronavirus disease 19 (COVID-19).

This is an investigational study.
Your participation in this research study is strictly voluntary. You may choose not to take part in this study without any penalty or loss of benefits to which you are otherwise entitled. The samples that you provide in this study for research purposes will be performed at no cost to you. You and/or your insurance provider will be responsible for the cost of any standard-of-care COVID-19 related testing.

Up to 1,500 people will take part in this study.

1. **STUDY PROCEDURES**

    COVID-19 is caused by the novel coronavirus, SARS-CoV-2. Researchers have developed a new diagnostic test to detect SARS-CoV-2 that could reduce the time it takes to receive a result, the cost associated with testing, and the laboratory infrastructure needed to support testing. Researchers are interested in evaluating the performance of the newly developed diagnostic.

    The nasopharyngeal swab you provide for SARS-CoV-2 screening will be tested using the approved diagnostic (standard of care [SOC]) at an MD Anderson lab, and this result will be communicated to you. A second nasopharyngeal swab, as well as self-collected cheek and nasal swabs and an oral rinse specimen will also be collected for research. These additional specimens will be used for research to evaluate the newly developed diagnostic test. If there is extra material left after the SOC test is performed, that may also be used for research testing.

The samples used for the research will be given a code number, and no identifying information will be directly linked to your samples. MD Anderson will make reasonable efforts to preserve your privacy but cannot guarantee complete privacy.

You will also be asked to complete questionnaires that will be sent to you by email or text message. The first questionnaire will be sent to you immediately after you enroll in the study to provide feedback about the different types of specimen collection. If you test positive for SARS-CoV-2, a second questionnaire will be sent one month after enrollment to provide information about the severity of your illness.

If you test positive for SARS-CoV-2, you will be referred for additional standard-of-care follow-up.

2. POSSIBLE RISKS

While on this study, you are at risk of discomfort from extra swabs being obtained, mouth/throat irritation, and taste changes. Side effects may vary from person to person. You are not at risk for additional known side effects.

This study may involve unpredictable risks to the participants.

3. POTENTIAL BENEFITS

Future testing efforts for COVID-19 and for future epidemic threats will be improved with what is learned from this study. There may be no benefits for you in this study.

4. OTHER PROCEDURES OR TREATMENT OPTIONS

You may choose not to take part in this study.

5. COSTS AND COMPENSATION

Payment for Injury
If you suffer injury as a direct result of taking part in this study, MD Anderson health providers will provide medical care. However, this medical care will be billed to your insurance provider or you in the ordinary manner. You will not be reimbursed for expenses or compensated financially by MD Anderson, or the study sponsor for this injury. You may also contact the Chair of MD Anderson's IRB at 713-792-2933 with questions about study-related injuries. By signing this consent form, you are not giving up any of your legal rights.

Certain tests, procedures, and/or drugs that you may receive as part of this study may be without cost to you because they are for research purposes only. However your health care plan and/or you may be financially responsible for the cost of care and treatment of any complications resulting from the research tests, procedures, and/or drugs. Standard medical care that you receive under this research study will be billed to your health care plan and/or you in the ordinary manner. Before taking part in this study, you may ask about which parts of the research-related care may be provided without charge, which costs your health care plan my pay for, and which costs may be your responsibility. You may ask that a financial counselor be made available to you to talk about the costs of this study.

There are no plans to compensate you for any patents or discoveries that may result from your participation in this research.

You will receive no compensation for taking part in this study.

ADDITIONAL INFORMATION

6. You may ask the researchers (Dr. Kathleen Schmeler, MD, at 713-745-3518) any questions you have about this study. You may also contact the Chair of MD Anderson's Institutional Review Board (IRB - a committee that reviews research studies) at 713-792-2933 with any questions that have to do with this study or your rights as a study participant.

7. You may also withdraw from participation in this study at any time without any penalty or loss of benefits. If you decide you want to stop taking part in the study, it is recommended for your safety that you first talk to your doctor. If you withdraw from this study, you can still choose to be treated at MD Anderson.

8. This study or your participation in it may be changed or stopped at any time by the study doctor, the U.S. Food and Drug Administration (FDA), the Office for Human Research Protections (OHRP), or the IRB of MD Anderson.

9. You will be informed of any new findings or information that might affect your willingness to continue taking part in the study and you may be asked to sign another informed consent and authorization form stating your continued willingness to participate in this study.

10. MD Anderson may benefit from your participation and/or what is learned in this study.

11. Authorization for Use and Disclosure of Protected Health Information (PHI):

    A. During the course of this study, MD Anderson will be collecting and using your PHI, including identifying information, information from your medical record, and study results. For legal, ethical, research, and safety-related reasons, your doctor and the research team may share your PHI with:
        *   Federal agencies that require reporting of clinical study data (such as the FDA, National Cancer Institute [NCI], and OHRP)
        *   The IRB and officials of MD Anderson
        *   Collaborators at Rice University
        *   Study monitors and auditors who verify the accuracy of the information
        *   Individuals who put all the study information together in report form

        Collaborators at Rice University will receive the research samples to evaluate the new diagnostic test.

    B. Signing this consent and authorization form is optional but you cannot take part in this study or receive study-related treatment if you do not agree and sign.

    C. MD Anderson will keep your PHI confidential when possible (according to state and federal law). However, in some situations, the FDA could be required to reveal the names of participants.

D. Once disclosed outside of MD Anderson, federal privacy laws may no longer protect your PHI.

E. The permission to use your PHI will continue indefinitely unless you withdraw your authorization by sending or faxing your request in writing. For MD Anderson participants, instructions on how to do this can be found in the MD Anderson Notice of Privacy Practices (NPP). You may contact the Chief Privacy Officer of MD Anderson at 713-745-6636 with questions about how to find the NPP. If you withdraw your authorization, the data collected up to that point can be used and included in data analysis, but no further information about you will be collected.

F. A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

[Patient signing consent content here – not sure what to do for mobile consent]

Evaluation of a Novel Point-of-care Diagnostic Test for SARS-nCoV-2
Protocol #: 2020-0318
Version Date: March 23, 2020

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1.0 Objectives

<u>Primary</u>:

1. To evaluate the clinical performance of a novel point-of-care diagnostic test for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-22), the virus that causes the disease called coronavirus disease 19 (COVID-19)

> **Commented [KP1]:** Ref:
> https://www.nature.com/articles/s41564-020-0695-z
> Pubmed id: 32123347

<u>Secondary</u>:

1. To compare the clinical performance of provider-collected nasopharyngeal samples with self-collected nasal swab, cheek swab and oral rinse samples using the novel SARS-nCoV-2 diagnostic test.
2. To measure viral load and evaluate the role of viral load in COVID-19 severity.

2.0 Background and Rationale

<u>Inaccessibility of testing for COVID-19</u>

There is an urgent need for widespread access to accurate, affordable diagnostic tools to respond to the global pandemic caused by COVID-19. Improved access to diagnostic testing is needed to help identify those patients and healthcare providers who should self-quarantine and to help guide public health efforts to control spread of the disease. However, there are many ongoing challenges associated with scaling up existing tests throughout the United States and globally. Clinics have reported shortages of test reagents, lack of trained personnel to perform testing, and, especially in rural and low-resource settings, lack of access to the expensive equipment needed to perform tests. Moreover, reports indicate that test results are often not available for 1-5 days, making it more challenging to appropriately care for patients who may be infected.

An affordable, accurate test that could be run by minimally trained personnel at the point-of-care could address these challenges. The goal of this protocol is to optimize and evaluate the clinical performance of a novel point-of-care diagnostic to detect the novel coronavirus (SARS-nCoV-2), the virus that causes the disease COVID-19. In addition, we will evaluate the performance of alternative sampling methods such as self-collected nasal swab, cheek swab and oral rinse samples which may have potential future use for at-home testing and decrease exposure of health care providers.

<u>Novel point-of-care test for COVID-19</u>

Together with industry partners, our team has developed a point-of-care nucleic acid test to detect SARS-nCoV-2. The test is simple to use, affordable, and can be rapidly manufactured for rapid deployment during this pandemic. It is a self-contained nucleic acid amplification test (NAAT) for SARS-nCoV-2 that fully integrates sample preparation, isothermal reverse transcription and amplification, and real-time detection into a simple and streamlined workflow.

Existing commercially available NAATs that rely on conventional polymerase chain reaction (PCR) or alternative isothermal amplification are too costly and complicated for use in many rural and low-resource settings, limiting long-term utility. In contrast, the platform we propose greatly reduces the cost and complexity associated with commercially available tests.

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While a number of researchers are developing simple low-cost NAATs for use in low-resource settings that leverage advances in isothermal amplification, there is not yet an effectively integrated point- of-care platform. Many prototype designs address a subset of the steps involved in the process of going from a clinical sample to an actionable test result, such as modules for sample preparation [1]–[8], amplification [4], [9]–[14], and/or amplicon detection [9], [14]–[17]. None to date has been able to combine all three steps into an effective, fully-integrated, sample-to-result platform. Although some recent publications have described integrated approaches, they do not yet achieve sufficiently sensitive detection levels and/or are not designed to be manufactured at scale. Moreover, amplicon contamination is a significant concern with any nucleic acid test that relies on amplification, and many low-cost strategies for isothermal amplification and detection are not fully enclosed, thus posing significant risk of workspace contamination and subsequent false positive results [18]–[20].

Together with an industry partner, Axxin Pty Ltd, we developed a new point-of-care nucleic acid test platform that combines sample preparation, isothermal amplification and real time detection into a single, integrated, affordable device (**Fig. 1**). Axxin is a leader in instrumentation development for point-of-care diagnostics. We have developed a COVID-19 test using the Axxin platform, which has a total instrument cost <$5,000 and can produce results within 30-45 minutes and does not required highly skilled laboratory technicians to run. The central technology is translated onto two Axxin platforms, shown by Workflow 1 and 2 in **Fig. 1**. The first platform shown in Workflow 1 has higher throughput, while the second platform shown in Workflow 2 has fewer manual sample transfer steps. We will evaluate both workflows.
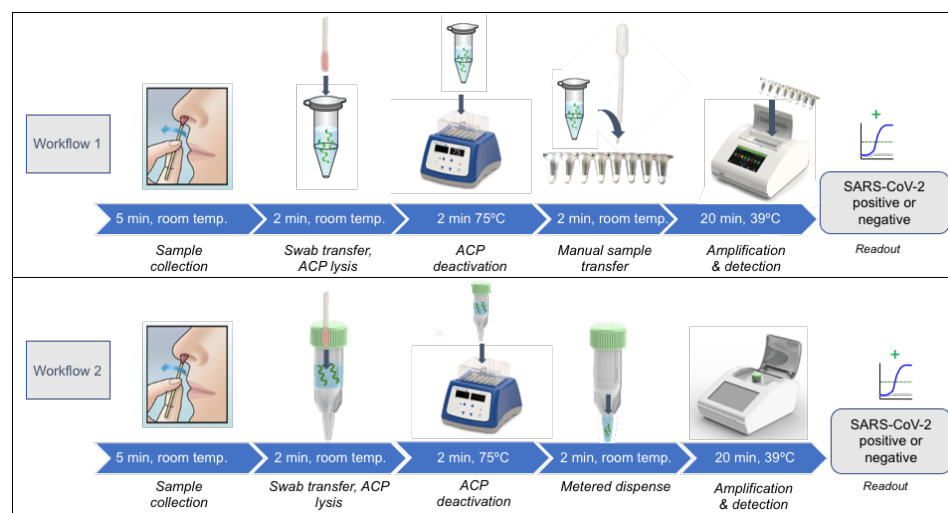
Fig. 1: *Developed point-of-care tests for COVID-19. Two anticipated workflows are shown involving the same central technology, but with higher throughput in Workflow 1 and with fewer manual sample transfer steps in Workflow 2.*

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Our collaborative team of investigators from Rice University and MD Anderson have extensive experience working together to develop and evaluate similar human papillomavirus (HPV) tests for cervical cancer screening both in the US and in low-resource settings such as Mozambique, Africa.

3.0 Patient Eligibility

Inclusion Criteria:

1. Age ≥ 18 years old
2. Qualifies for SARS-nCoV-2 testing at MD Anderson or affiliated sites (may include MD Anderson patients and employees) according to institutional criteria at time of enrollment
3. Signed informed consent and ability to perform protocol-required activities
4. Able to speak and read English or Spanish

Exclusion Criteria:

1. Patient or provider decision not to perform SARS-nCoV-2 testing

4.0 Study Design

This is a cross-sectional study involving individuals 18 years of age or older who are undergoing COVID-19 testing at MD Anderson and affiliated sites.

5.0 Treatment Plan

The study will enroll up to 1,441 individuals undergoing SARS-nCoV-2testing.

Individuals (patients and employees) undergoing SARS-nCoV-2testing at MD Anderson and affiliated sites meeting the inclusion criteria will be invited to participate in the study. They will be asked to provide informed consent. Basic information will be collected including date of birth, gender, symptom burden and underlying medical conditions (Appendix A). Participants will undergo two nasopharyngeal swab sample collections by a health care provider. The participant will then be provided instructions and undergo self-collection of a nasal swab, cheek swab and oral rinse sample. The oral rinse sample collection will consist of ten milliliters of mouthwash and will be swished in the oral cavity for 15 seconds, gargled for 15 seconds, and expectorated into a specimen cup.

One of the nasopharyngeal swabs will be sent for standard-of-care SARS-nCoV-2 testing at the MD Anderson Molecular Diagnostic Lab by RT-PCR or a suitable substitute reference method. The second nasopharyngeal swab as well as the self-collected nasal swab, cheek swab and oral rinse samples will be transferred to Rice University for testing on the novel diagnostic platform. The specimens will be de-identified and transported using a vendor-approved courier. The research specimens will be batched and picked up from a designated access point at MD Anderson and transferred to Rice University on a daily basis or at regularly scheduled intervals based on specimen volume and/or courier availability. Any remaining material from the standard of care specimen may also be used for research testing.

All samples will be processed in the lab at Rice University. They will be transferred into a 15 ml tube. Specimens will be refrigerated until processing or freezing at -80°C. Testing will include validation of the performance of a novel point-of-care diagnostic for SARS-nCoV-2. Test

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characteristics will be optimized for accuracy and results of the novel test will be compared to the gold standard. In addition, viral load will be measured.

Participants will be asked to complete two questionnaires following enrollment. These questionnaires will be sent via email or text message (based on the participant's preference) using REDCap. The first questionnaire will be sent immediately following specimen collection to provide feedback regarding the provider-collected vs. self-collected samples (Appendix B). If no response is received within 24 hours, a repeat request will be sent. A second questionnaire will be sent to participants who tested positive for SARS-nCoV-2 by either the standard or care test or novel diagnostic test conducted at Rice University. It will be sent one month following study enrollment and will include questions regarding disease severity and outcomes (Appendix C). If no response is received, a repeat request will be sent two days later.

Participants who test positive per the standard of care test will be contacted according to standard procedures at MD Anderson. Participants who test negative on the standard of care test but positive on the Rice test will be contacted and asked to return for a repeat standard of care test.

6.0 Study Visits

This study will include only a single in-person visit with two follow-up questionnaires (immediately following specimen collection and one month later if either of the tests were positive). In addition, the participant's medical record at MD Anderson will be reviewed by the study team for standard of care COVID-19 test results as well as demographic, clinical and outcome information as available.

7.0 Statistical Considerations

Sample Size:

Testing 1,441 individuals will allow us to estimate the sensitivity of the test with a margin of error of at most 5% if the prevalence of SARS-nCoV-2 is at least 20% and the sensitivity is at least 75%. Testing 1,000 individuals will allow us to estimate the specificity of the test with a margin of error of at most 3% if the prevalence of SARS-nCoV-2 is at least 20% and the specificity is at least 75%. Therefore, we will test 1,441 individuals to achieve the desired precision in our estimates of sensitivity and specificity. These sample size estimates were taken from Tables 1 and 2 in the paper by Hajian-Tilaki [21].

Analysis:

We will use summary statistics to describe age and gender of the individuals participating in this study overall and by standard-of-care test result (positive, negative).

We will use the standard-of-care (RT-PCR) COVID-19 test at the MD Anderson Molecular Diagnostic Lab result for SARS-nCoV-2 as the true result to estimate sensitivity and specificity of the novel point-of-care diagnostic test. We will provide these estimates of sensitivity and specificity of the novel point-of-care diagnostic test separately for each of the following:

- the provider-collected nasopharyngeal samples,
- the self-collected nasal swab,
- the self-collected cheek swab, and
- the oral rinse samples.

We will use descriptive statistics to summarize viral load metrics from the standard-of-care (RT-PCR) COVID-19 test and from each of the point-of-care tests enumerated above. We will use logistic regression methods to model progression to severe disease as a function of viral load for each of these tests.

8.0 Data and Protocol Management

Registration: All patients enrolled in this study will be registered in the Clinical Oncology Research System (CORE) at The University of Texas, MD Anderson Cancer Center.

Data Collection and Confidentiality Procedures: Study data will be collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at MD Anderson. REDCap (www.project-redcap.org) is a secure, web-based application with controlled access designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless downloads to common statistical packages; and 4) procedures for importing data from external sources. In the case of multi-center studies REDCap uses Data Access Groups (DAGs) to ensure that personnel at each institution are blinded to the data from other institutions.

REDCap (https://redcap.mdanderson.org) is hosted on a secure server by MD Anderson Cancer Center's Department of Research Information Systems & Technology Services. REDCap has undergone a Governance Risk & Compliance Assessment (05/14/14) by MD Anderson's Information Security Office and found to be compliant with HIPAA, Texas Administrative Codes 202-203, University of Texas Policy 165, federal regulations outlined in 21CFR Part 11, and UTMDACC Institutional Policy #ADM0335. Those having access to the data file include the study PI and research team personnel. Users are authenticated against MDACC's Active Directory system. External collaborators are given access to projects once approved by the project sponsor. The application is accessed through Secure Socket Layer (SSL). All protected health information (PHI) will be removed from the data when it is exported from REDCap for analysis. All dates for a given patient will be shifted by a randomly generated number between 0 and 364, thus preserving the distance between dates. Dates for each patient will be shifted by a different randomly generated number. Following publication study data will be archived in REDCap.

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APPENDIX A. Participant Demographic Information

1. Name
2. Date of birth
3. Gender
4. Contact information:
   - Email
   - Telephone number
   - Alternate telephone number
   - Preferred method of contact:
     - email, text message, telephone call, other
   - Emergency contact – email and telephone number
   - Preferred language:
     - English or Spanish
5. Employee – Y/N
   - Patient facing – Y/N
     - Position – doctor, nurse, technician, other
6. Do you have any of the following medical conditions?
   - Cancer – Y/N
   - Heart disease – Y/N
   - Respiratory conditions – Y/N
   - Diabetes – Y/N
   - Other
7. Which of the following symptoms are you currently experiencing?
   - Fever
   - Cough
   - Shortness of breath
   - Sore throat
   - Other
8. Have you been exposed to someone who tested positive for COVID-19? – Y/N

Appendix B. Participant Survey Regarding Testing and Specimen Collection Methods

1. Which one of the three sample collection methods did you prefer today?
   - The provide-collected nasopharyngeal swab
   - The self-collected cheek swab
   - The self-collected mouth rinse
2. How comfortable was the provider-collected nasopharyngeal swab?
   - Scale of 1-10 (with 1 being very uncomfortable to 10 being very comfortable)
3. How comfortable was the self-collection of the nasal swab?
   - Scale of 1-10 (with 1 being very uncomfortable to 10 being very comfortable)
4. How comfortable was the self-collection of the cheek swab?
   - Scale of 1-10 (with 1 being very uncomfortable to 10 being very comfortable)
5. How comfortable was the self-collection of the mouth rinse?
   - Scale of 1-10 (with 1 being very uncomfortable to 10 being very comfortable)

Appendix C. Participant Follow-up Questionnaire

1. Were you hospitalized due to COVID-19 illness? - Y/N
   - How many days were you hospitalized for?
   - Did you require admission to the intensive care unit (ICU)?
     - How many days?
   - Did you require intubation?
     - How many days?
2. Are you currently back to normal activities? Y/N
3. If MD Anderson employee
   - Are you currently back at work?

DEVELOPMENT OF A SEROLOGIC TEST FOR COVID19

OUTLINE
RESEARCH WORKING GROUP MEMBERS
KEY POINTS
SUMMARY
ONGOING EFFORTS
PROPOSAL FOR SEROLOGY TESTING

WORKING GROUP
SEROLOGIC TEST DEVELOPMENT FOR COVID19 RESEARCH
Nadim Ajami, PhD
Eleonora Dondossola, PhD
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Raghu Kalluri, MD
Jason Bock, PhD
James Kelley, MD PhD
Hector Alvarez, MD

KEY POINTS
- The development of a serologic test (ELISA) to detect circulating antibodies against SARS-CoV-2 is critical for the fight against COVID19 by identifying individuals who have overcome the infection and are presumed immune.
- Learnings from animal studies using SARS-CoV-2 and previous coronavirus outbreaks (i.e. SARS and MERS) shows that infection can result in protective immunity and in the case of SARS and MERS for up to three years.
- There are quantitative serologic tests that are currently available commercially, however FDA-approved tests to date are not quantitative. Nonetheless, it is important to capitalize on available data to help our workforce and our patients.

SUMMARY
A serologic test for COVID19 detects the presence and measures the amount and type of antibodies in the blood elicited after the infection, irrespective of the presence of clinical symptoms. Although this test has limitations in diagnosing active COVID19, it plays a critical role in the fight against it by identifying individuals who have overcome the infection and are presumed immune. This test will help us determine which individuals are no longer susceptible to COVID19 and can return to work, and who can donate plasma that could serve as a possible treatment for severe cases - both high priorities for our institution. In addition, validated serologic tests will be crucial for patient contact tracing, identifying the viral reservoir hosts, and epidemiologic studies as we have witnessed in past outbreaks.

There are many formats in which serologic tests can be carried out but in this case an enzyme-linked immunosorbent assay (ELISA) is the most optimal option. ELISAs provide a relatively quick (1 day turnaround time) and inexpensive readout of antigen-antibody interaction that can

be quantitative and specific for viral antigens and human antibodies. Previous work with SARS and MERS outbreaks, both caused by coronaviruses, identified an ELISA method that provided high sensitivity to detect the virus and was more efficient than other approaches.

As other institutions have noted so far, epidemiologic studies are urgently needed to help uncover the burden of disease, in particular the rate of asymptomatic infections, and to get better estimates on illness and death. These epidemiologic studies can help identify the extent of virus spread in households, communities, and specific settings like our hospital and research buildings, which could help guide control measures. In the near future, serologic assays will be needed for evaluation of results of vaccine trials.

To date, only one assay has been approved by the FDA (Cellex, lateral flow assay) to detect circulating antibodies against SARS-CoV-2, but this assay does not offer a quantitative readout nor can it distinguish the type of antibody measured. Given the lack of available solutions, it is imperative that our institution invests in developing a serologic test to be used for preclinical purposes with the potential of implementation in the CLIA laboratory.

To this end, we have engaged with external groups (Florian Krammer, Mount Sinai and Pedro Piedra, Baylor College of Medicine) to source reagents and support collaborators in the development of serologic tests. In addition, reagents and methodologies have been developed at our institution (Dr. Raghu Kalluri) prior to the halt on research activities that can be used to produce a serologic test.

IMMEDIATELY AVAILABLE ASSAYS

CELLEX:
https://www.fda.gov/media/136625/download

The Cellex qSARS-CoV-2 IgG/IgM Rapid Test is a lateral flow immunoassay intended for the qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in serum, plasma (EDTA, citrate) or venipuncture whole blood specimens from patients suspected of COVID-19 infection by a healthcare provider approved by the FDA.

The qSARS-CoV-2 IgG/IgM Rapid Test is an aid in the diagnosis of patients with suspected SARS-CoV-2 infection in conjunction with clinical presentation and the results of other laboratory tests. Results from the qSARS-CoV-2 IgG/IgM Rapid Test should not be used as the sole basis for diagnosis. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. 263a, to perform moderate and high complexity tests.

GENALYTE

Genalyte specializes in rapidly deploying mobile, on-site laboratories that deliver most frequently ordered tests in ~20 minutes. Genalyte operates CLIA/CAP accredited laboratories and in addition to providing instruments and kits, they offer a mobile STAT labs solution.

Their serologic test for COVID19 is offers results for entire panel (two spike proteins, one nucleocapsid protein, detects IgM and IgG) in 15 minutes and can test 4 patients simultaneously, up to 16-plex. Requires <250ul whole blood, via finger prick or venipuncture and can be cloud-connected for remote clinical oversight. The company is currently pursuing Emergency Use Authorization with the FDA.

ASSAYS AVAILABLE IN NEAR TERM (1-4 WEEKS)

WORK GROUP: Drs. Nadim Ajami, Eleonora Dondossola, MDA; Pedro Piedra, Baylor College of Medicine; Florian Krammer, Mount Sinai

Goal: Develop a quantitative ELISA to detect IgM and IgG in human plasma/serum against SARS-CoV-2 spike protein and the receptor binding domain (**RBD ELISA**)

Reagents: Proteins and plasmids expressing SARS-CoV-2 spike protein and RBD have been shared by Dr. Florian Krammer (Icahn School of Medicine, Mount Sinai; several in-house tests have been developed worldwide based on these reagents, including multiple Stanford labs); and shared with Dr. Piedra at BCM to develop an ELISA under RUO settings and to be later transferred to a CLIA environment. 12.5 µl of plasma or serum needed/test (2 wells, in duplicate, 6.25 µl/well)

Results: Krammer's antigen was successfully recognized by anti-CoV2 antibodies, while commercially available antigens were not (possibly due to production in mammalian cells that support better folding and post-translational modifications), see table below:

		mAb/polyclonal/human serum								
	Subunit	Mouse CoV-1-S1 (ThermoFisher)	Rabbit CoV-2-S1 (SinoBiological)	Rabbit CoV-1-S (FDA)	Rabbit CoV-2-S (FDA)	Rabbit Neg control serum (FDA)	Flor (human serum)	PAP (human serum)	204490769 (human serum)	204490775 (human serum)
SARS-CoV-2	S1 (230-01101,RayBiotech)	0.3,0.2	0.3,0.1	0.1,0.1	0.1,0.1	0.1,0.1	1.3,1.7	1.8,2.0	1.3	1.1
	RBD (230-01102,RayBiotech)	0.2,0.2	0.2,0.1	0.1,0.1	0.1,0.1	0.1,0.1	3.1(1:2) 1.7,2.0	1.8,1.9	1	0.7
	RBD (Florian Krammer)	0.1,0.1	>4.0,>4.0	0.1,0.1	3.8,>4.0	0.1,0.1	0.2 0.2,0.2	0.3,0.2	0.1	0.1

OD values of SARS CoV-2 ELISA

ASSAYS AVAILABLE IN THE LONGER TERM (4 WEEKS OR MORE)

WORK GROUP: Dr. Raghu Kalluri

Goal: Develop an ELISA to detect IgM and IgG in human plasma/serum against SARS-CoV-2 spike protein (**SPIKE ELISA**)

Reagents: Recombinant soluble SARS-CoV-2 Spike ectodomain protein production in 293T cells was achieved.

WORK GROUP: Drs. Kathleen Schmeler (MDA), and Rebecca Richards-Kortum (Rice University)

Goal: Develop a point-of-care test (**PoC**)

Reagents: The PoC is a paper-based test for viral antigen and antibody detection for low-resource settings that provides a semi-quantitative readout. Dr. Richards-Kortum research and teaching focus is on the development of low-cost, high-performance technologies for remote and low-resource settings. About to receive plasmids to start production of Spike proteins

RESEARCH PROPOSAL FOR TESTING OUR WORKFORCE AND PATIENTS

COVID19 ANTIBODY RAPID SCREENING ALGORITHM FOR PATIENTS, HEALTHCARE AND ESSENTIAL WORKERS INCLUDING THOSE IN HIGH-RISK AREAS NOT PRESENTING SYMPTOMS OF DISEASE

PRIORITY LEVEL 1 — Healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

PRIORITY LEVEL 1 — Patients recovered from COVID19

PRIORITY LEVEL 2 — Patient-facing HCWs not presenting symptoms

PRIORITY LEVEL 3 — Essential workers and others in high-risk areas not presenting symptoms

PRIORITY LEVEL 3 — Blood products

Serology → Negative → Not presumed immune — Retest in 45 days in the absence of symptoms (N/A for blood products)

Serology → Positive

RT-qPCR (MolDx Lab) → Negative → Serology → Negative → Retest in 21 days → Negative → Not presumed immune

RT-qPCR (MolDx Lab) → Positive → Isolation — Retest in 14 days

Serology → Positive (If prior serology test was positive) → Presumed Immune

Retest in 21 days → Positive → Presumed Immune

IN-HOUSE TESTING: PRIORITY LEVELS 1 & 2
SEND-OUT: PRIORITY LEVELS 2 &3

Phase I

1. Reagent Development & Assay Validation

· *Activities:* Produce recombinant RBD to keep on performing RBD ELISA ; produce recombinant Spike protein to perform SPIKE ELISA; include a robust QC panel to use for internal development and share with collaborators; develop and qualify a serological test using a limited (<10 de-identified patient samples with confirmed molecular positive and negative for CoV-19)

· *Timeline:* 1-4 weeks
· *Resources***:** For 1,000 research trial tests tests a day, we will need ~6 FTEs (5 individuals for production, testing and data reporting and 1 supervisor)
· *Equipment***:** ELISA plate readers are available
· *Estimated cost and supplies***: ~**$1-2 per test, assuming a standardized test for 10,000-20,000 samples, to cover reagent expenses

2. Assay Utilization
· *Activities:* Run an initial epidemiologic study on ~250 employees from MDACC research (~10%) modeled after the national NAIAD study and include a questionnaire to determine prior symptoms. This would help us understand the existing exposure level for our employees and help quantify the risks of opening to future exposures.This would be run by Research personnel on research staff, with no impact on the clinic
· *Timeline:* 3 weeks
· *Effort:* 6 FTEs

Phase II
Based on the results of Phase I and the then current landscape, determine if we should extend the testing to the wider research community as an input into a staged, risk-based, informed, back-to-plan for research.

TITLE: **Evaluating the seroprevalence of SARS-CoV02 antibodies in an asymptomatic or subclinical population at MD Anderson Cancer Center**

Study Chair: Nadim Ajami
Co-Chairs: Eleonora Dondossola
Collaborators: Florian Krammer, Pedro Piedra
Department: Genomic Medicine
Phone:
Unit:

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Commented [MOU2]: Carrie Daniel MacDougall
Paul Scheet
Alex Lazar

Commented [MOU3]: Non-MDA - can we do this and is it smart?

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Participant Population

Expected age range of participants:
18 and above

Will this include embryonic stem cells?
No

Does this research include MDACC employees as participants?
Yes

Total expected number of participants is 5,000 from 04/01/2020 through 04/01/2021. These are COVID19 patients or persons under investigation who undergo laboratory testing for COVID19 and where blood samples are drawn for standard of care procedures.

Specimen Type and Collection Type:
Residual blood serum samples

Total # of Specimens, Volume, Frequency:

One sample per patient or as many as available up to 5,000 patients' samples.

Commented [MOU4]: How does this sound?

List the source of specimens/data (Select all that apply):
The data of patients will be collected from the electronic medical record (Epic).

Data Profile

Types of data points that will be collected include:
Names or initials, medical record number, date of birth, gender, medications, medical comorbidities, smoking history, dates of surgery and other treatments at MD Anderson including intravenous or oral antibiotics administered and/or prescribed, dates of followup, results of imaging/pathology/labwork, date of recurrence/progression, and date of death.

Specimens/data sharing
Specimens/data will NOT be shared with any entity, person, or organization outside of MD Anderson

Confidentiality

Will there be a link to identify subjects?
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Will identifiable data be made available to anyone other than the PI?
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Compensation
Will subjects receive compensation (e.g. water bottles, t shirts, gift cards), or reimbursements (e.g., gas cards for travel, parking vouchers, bus fare, or hotel reimbursement?)
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Informed Consent Considerations

Please select the appropriate response(s) regarding the informed consent process for this study (you may select more than one)
Protocol staff will obtain prospective written consent

1.0 Objectives

The **primary aim** of this research study is to **determine the seroprevalence of antibodies against SARS-CoV-2, the causing agent of COVID19, among our patient population and workforce**. This study represents the initial step to determine the rate of seropositiviy in our community. The data resulting from this study will be used to guide the development of larger ongoing study to determine the seroprevalence of SARS-CoV-2 antibodies in our population over the next 5 years.

Outcome Measures:
Primary:
- Determine the extent of SARS-CoV-2 infection in our community ~~as determined~~by means ~~by~~ of seropositivity.

Secondary:
- Determine the cumulative incidence of infection in our patient population and workforce.
- Determine the isotype of antibodies raised against surface antigens of SARS-CoV-2

2.0 Rationale

Severe acute respiratory disease coronavirus 2 (SARS-CoV-2) has caused a pandemic known as **Co**rona**vi**rus **D**isease 20**19** (COVID-19). Patient's symptoms range from no detectable signs of infection, to fever, cough, acute respiratory distress syndrome and pneumonia that can lead to death in 1.4% of cases[1] across the general population and up to 8-20% in more vulnerable populations (i.e. >70 years of age, presence of coexisting disorders, including cancer)[2]. Several molecular assays are currently available to identify positive patients by directly detecting viral RNA in respiratory samples through real time reverse transcriptase-PCR. However, these assays have to be performed during a narrow window following infection otherwise the virus cannot longer be detected. Thus, testing of asymptomatic people has been challenging due to resource management and prioritization of symptomatic people, leaving many undiagnosed.

Irrespective of the ~~presence~~ absence of symptoms (which have been reported as high as 88%[3]), the infection leads to the generation of antibodies that can be detected in blood for months or longer following infection, offering an opportunity to determine the magnitude of cases via serologic testing. Although detecting antibodies against SARS-CoV-2 is indicative of prior ~~infection~~exposure, it has diagnostic limitations for the acute stages of the disease as antibodies are generated 7-28 days after infection[4]. ~~Thus, testing of asymptomatic people has been challenging due to resource management and prioritization of symptomatic people, leaving many undiagnosed~~ Therefore, a serologic test for COVID-19

~~A serologic test for COVID19 detects the presence and measures the amount of antibodies in the blood elicited after the infection, irrespective of the absence of clinical symptoms~~

(which have been reported as high as 88%[4]) and would complement PCR-based results to calculate the proportion of infected individuals among our community of patients and our workforce. Identifying those who have overcome the infection is needed to estimate illness prevalence in the overall population and have great implications in our understanding of how immunity develops, particularly in cancer patients.

Although limited information is available for SARS-CoV-2, protective immunity has been shown in primates after re-challenge to CoV2, with no evidence of viral loads and viral replication in all primary tissue compartments at five days post-reinfection[5]. In another study, and in limited number of cases sseroconversion (IgG and IgM) in SARS-CoV-2 patients has been shown to occur by 7 days in 50% of

Figure 1. Adapted from Wölfer et al.[4] Viral load kinetics, seroconverstion, and clinical observations in individual cases. Panels A to I correspond to 9 different cases studies. Dotted line, limit of quantification were performed in duplicate and the data presented are means of results. Experiment obtained by two laboratories independently.

patients individuals and 14 days in all of them, as reported[4] (Fig. 2([4])). Similar results were confirmed by others ([6]) and evidence continues to grow. A more comprehensive study (n=175) detected neutralizing antibodies in >95% of patients positive to CoV-2 using a neutralization assay (Fig. 2)[7]

There are many formats in which serologic tests can be carried out but in this case an enzyme-linked immunosorbent assay (ELISA) is the most optimal option. ELISAs provide a relatively quick (>24 hours turnaround time) and inexpensive readout of antigen-antibody interaction that can be

Figure 2. Adapted from Wu et al.[7] Titers of neutralizing Abs against VSV, SARS-CoV, and SARS-CoV-2 pseudovirus in 26 COVID-19 recovered patient plasma. More than 95% developed antibodies above the detection limit (50 OD).

quantitative and specific for viral antigens and human antibodies. Previous work with SARS and MERS outbreaks, both caused by coronaviruses, identified an ELISA method that provided high sensitivity to detect the virus and was more efficient than other approaches. No cross-reactivity was shown to other coronaviruses (OC43, HKU1, 229E, NL63) in ELISA assays recently developed to test SARS-CoV-2 (Fig. 3)[6,8,9]. With this novel coronavirus, initial seroprevalence in the population is assumed to be negligible due. Therefore, surveillance of antibody seropositivity in a population can allow inferences to be made about the extent of infection and about the cumulative incidence of infection in the population.

Fig. 4. Adapted from Okba et al. [6] 87%–100% of serum samples in control cohorts (blood donors, non-CoV infection, HCoV) were seropositive for endemic HCoVs (HCoV-HKU1, HCoV-OC43, HCoV-NL63, and HCoV-229E), as determined by the S1 protein microarray. No antibody cross-reactivity was shown with SARS-CoV-2.

Based on this evidence, eEpidemiologic studies are urgently needed to help uncover the burden of disease, in particular the rate of asymptomatic infections, and to get better estimates on illness and death. These epidemiologic studies can help identify the extent of virus spread in households, communities, and specific settings like our hospital and research buildings, which could help guide control measures. In the near future, serologic assays will be needed for evaluation of results of vaccine trials.

Hypothesis: we hypothesize that SARS-CoV-2 induces seroconversion in infected individuals stimulating the production of circulating antibodies against the virus.

3.0 Eligibility of Subjects

Inclusion: Adult subjects (≥18 years) with a laboratory confirmed diagnosis of COVID19 or under investigation for possible COVID19:
- Residual blood serum samples from laboratory confirmed COVID19 cases or from presumed cases (persons under investigation) will be obtained from the Institutional Tissue Bank (ITB) at MD Anderson Cancer Center

Exclusion: A subject will not be eligible for inclusion in this study if any of the following criteria apply:
- Samples kept in suboptimal conditions will be excluded from this study.

4.0 Research Plan and Methods

The seroepidemiological investigation for COVID19 virus among MD Anderson is intended to provide key epidemiological and serologic characteristic of the disease. The model employed will follow a repeated cross-sectional investigation in which samples will be drawn from a specific population (i.e. MD Anderson Cancer Center patients and potentially the workforce) but not necessarily from the same individuals each time. The timing of the study will depend on our ability to return to the research laboratories.

Blood serum samples collected from individuals with confirmed laboratory results for COVID19 and others presumed to have the disease but not diagnosed (persons under investigation) will be included. These samples are collected as part of standard of care procedures and residual volumes are stored at the Institutional Tissue Bank (ITB). We will use those samples to evaluate the presence and the quantity of antibodies against SARS-CoV-2 to determine seroposivitiy rates.

The immunoassay that will be used to study the seroprevalence of antibodies against SARS-CoV-2 is being developed in collaboration with Florian Krammer, Professor of Vaccinology at the Department of Microbiology at the Icahn School of Medicine at Mount Sinai, the Principal Investigator of the Sinai-Emory Multi-Institutional Influenza Vaccine Innovation Center (SEM-CIVIC), and member of the NIH-funded Centers for Excellence in Influenza Research and Surveillance (CEIRS). The IgG ELISA developed by Dr. Krammer got Emergency Use Approval by the FDA on April 16 (https://www.fda.gov/media/137032/download). Dr KrammerHe has shared reagents and a protocol for the development of the assay with the Chair and Co-Chair of this study and such reagents are being further shared with Dr. Pedro Piedra at Baylor College of Medicine who is leading the local implementation of Dr. Krammers' protocol. Dr. Piedra is a Professor in the Department of Molecular Virology and Microbiology and his research is focused on influenza virus, respiratory syncytial virus, adenovirus viruses, human metapneumovirus, and the recently described SARS-CoV-2.

The surface glycoprotein of the virus, termed spike (S) protein, mediates attachment of the virus to human cells via its receptor binding domain (RBD) and subsequentmediates fusion of viral and cellular membranes[10]. Antibodies that bind to the spike protein, and especially to the RBD domain, can neutralize coronaviruses. This assay uses different recombinant

spike protein preparations as antigen for the ELISA. It has been reported that individuals not exposed to SARS-CoV-2 are completely naïve for the spike protein and show ~~limited~~ no reactivity in an ELISA[6,8,9] test. It is, therefore, possible to distinguish between exposed/immune and naïve people.

As described by Amanat et al[8], a two-step ELISA protocol for high throughput screening of human serum samples for antibodies binding to the spike protein of SARS-CoV-2 will be implemented. In this a two-step ELISA. ~~in which T~~the first step includes relatively high throughput screening of samples in a single serum dilution against recombinant~~the~~ RBD produced in mammalian cells (which expresses very well and therefore ~~there is~~leads to a higher ~~typically more~~ protein ~~available~~yield). This is followed by a second step in which positive samples from the first step undergo a confirmatory ELISA against the full length spike protein (which is harder to purify, therefore there is usually less available). For the second step a dilution curve is performed. The throughput of this assay, if only one operator is available, allows to perform screening ELISAs (760 samples/10 plates per run) and confirmatory ELISAs ~~can be run~~ in (140 samples/10 plates per run) in a single day. Negative controls include serum pools of serum taken before 2020. Positive controls are convalescent samples from COVID19 patients or monoclonal antibodies (mAbs) like CR30229[11,12]. If no human sera or mAbs are available, mouse mAbs, mouse sera against SARS-CoV-2, order animal sera against SARS-CoVo2 or anti-his tag antibodies (the recombinant proteins used as antigens are his-tagged) can be used. However, in this case a different secondary antibody for the species from which the primary antibody is derived is needed for the positive control. The positive control exceeds an OD_{490} of the negative control plus 3 standard deviations of the negative controls up to, at least, a 1:150 dilution. ELISAs can be run with both serum and plasma.

4.1 Subjects

Patients undergoing laboratory testing for COVID19 diagnosis at MD Anderson Cancer Center from 04/1/2020 to 04/01/2021 will have their blood drawn for standard of care procedures. Residual samples collected in serum separator tubes (SST, i.e. gold or red/gray) or serum tubes (i.e. red cap) will be stored at the Institutional Tissue Bank and used for this research.

MD Anderson staff including healthcare workers and research staff presenting no symptoms for COVID19 will be enrolled to determine seroprevalence of antibodies against SARS-CoV-2. Samples will be collected in a similar way as for patients undergoing standard of care testing as explained above.

> **Commented [MOU5]:** This is not going to be residual samples, is it ok anyway?

4.2 Sample Collection

Blood serum residual samples obtained from the Institutional Tissue Bank from laboratory confirmed COVID19 cases and persons under investigation (PUIs) will be assayed to detect and quantify antibodies against the viral receptor binding domain (RBD) of the spike protein of SARS-CoV-2.

> **Commented [MOU6]:** Here you do not refer to MDA workforce

Potential Harms/Adverse events:
No potential harms or adverse events are anticipated other than the very minimal risk associated with blood draws. The primary benefit of the study is indirect in that data collected will help improve our understanding of COVID19.

Safety Monitoring:
This study will be monitored by the MDACC Data and Safety Monitoring Board (DSMB).

ELISA testing should be carried out in a facility with at least biosafety level 2 (BSL-2) capacity. All personnel involved in the investigation need to be trained in infection prevention and control procedures (standard contact and droplet precautions, as determined by national or local guidelines). These procedures should include proper hand hygiene and the correct use of surgical masks, if necessary, not only to minimize their own risk of infection when in close contact with infectious material, but also to minimize the risk of spread among other participants in the investigation.

4.3 Testing

A two-step enzyme-linked immunosorbent assay (ELISAs) based on recombinant antigens derived from the spike protein of CoV-2, including its receptor binding domain (RBD) and full length spike (S) protein will be used. Such antigens were used for testing 59 negative human control sera preCOVID-19, including people with previous confirmed coronavirus exposure (e.g. NL63) and four samples from CoV-2 positive patients. ELISAs proved sensitive and specific and allowed for identification of positive patients using human plasma/serum as early as three days post-symptom onset (12.5 µl of plasma or serum needed/test; 2 wells, in duplicate, 6.25 µl/well). As an advantage, these assays do not require handling of infectious virus, are amenable to scaling and only few microliters of plasma/serum are needed. The assay will have sensitivity of >95% and specificity of >95% and pre-pandemic sera are used as controls to test cross reactivity to other coronavirus. As mentioned, this assay got Emergency Use Approval by the FDA.

Residual samples will be obtained from the Institutional Tissue Bank for testing. Both serum and plasma samples can be tested for the presence of antibodies against SARS-CoV-2.

> **Commented [MOU7]:** What about workforce???

5.0 Statistics and Justification of Sample Size

Sample size justification

> **Commented [MOU8]:** incomplete

For epidemiological studies it is important to capture a sample that is representative of the population at risk to understand the extent of the virus spread and to reduce the margin or error. Due to the fact that this is a virus newly introduced to humanity, initial seroprevalence

in the population is assumed to be minimal thus the entire population of patients and workforce is considered at risk.

Data analysis

Several epidemiological parameters will be evaluated including age-specific cumulative incidence which is defined as the proportion of individuals per age strata that are seropositive for COVID19 infection taking into account any difference the age stratification of the participants and the overall population. In addition, the proportion of asymptomatic cases over total number of confirmed cases at MD Anderson will be determined. Specifically, in cancer patients, we will quantify the presence of antibodies in confirmed COVID19 cases and compare it to the general population, and for patients with repeated samples, the change in levels of SARS-CoV-2 antibodies will be determined. Lastly, the case fatality ratio defined as the proporteion of individuals with fatal outcome for COVID19 infection will be established. This may require extended follow-up to determine the outcome of infection among those tested.

6.0 Procedure to Obtain Informed Consent

> **Commented [MOU9]:** Do we need IC if these are residual samples?

7.0 Data Confidentiality

Data will be available to the PI and people directly involved with the collection and analysis of data related to this project. IRB approval will be obtained for any exchange of data within and outside of MD Anderson.

Collection of Identifiers:
Identifiers (name, dates, phone number, email address and MRN) will be collected. Identifiers (other than dates) will be replaced by study numbers in the analytic file. The key linking these numbers will be retained in a locked file or stored on an encrypted server behind the firewall, by the investigator's designated personnel. Dates will be retained as a limited data set. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval.

Training of Personnel:
All MD Anderson personnel will be fully trained to maintain the patient health information confidentially. Training will be documented as required by institutional policy.

Data Storage:
The PI and research staff will attempt to minimize risk through only storing information containing subject identifiers in locked file storage, and on password-protected computers, on encrypted servers behind an institutional firewall and according to current institutional

and federal data security requirements. In addition, access to patient identifiers will be limited to the minimum number of necessary research personnel, and only to those research personnel directly involved with obtaining patient information and assigning random study identifiers. Keys containing information linking study subjects to personal identifiers will be maintained in locked storage for paper records or behind institutionally approved firewall and electronic security measures for electronic keys, and available ONLY to the PI and research personnel directly involved in creating random study identifiers. Information containing subject personal identifiers will not be removed from MD Anderson Cancer Center without IRB approval and will not be shared in publications or reports concerning this research study.

Data Sharing:
Study data will not be shared with any individuals or entities that are not involved in the study. No identifying information will be shared with outside collaborating sites or outside collaborating research staff without prior IRB approval and a data use or material transfer agreement has been implemented. Sharing of data will be done after approval of the PI and only by secure mechanisms, as approved by MD Anderson Information Security.

Final Disposition of Study Records:
These data will be used for this research study. Data that is in hard-copy form will be retained on site until the study is terminated, and may be stored indefinitely, per institutional standards, in long-term off-site storage with an MD Anderson approved, secured contract site. Electronic data will be retained indefinitely on MD Anderson servers behind the institutional firewall. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval. Study data and paper records will not be destroyed but will be retained permanently.

8.0 References

1.   Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020.
2.   Onder G, Rezza G, Brusaferro S. Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *JAMA* 2020.
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12.   Tian X, Li C, Huang A, et al. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerg Microbes Infect* 2020; **9**(1): 382-5.

TITLE: Evaluating the seroprevalence of SARS-CoV02 antibodies in an asymptomatic or subclinical population at MD Anderson Cancer Center

Study Chair: Nadim Ajami
Co-Chairs: Eleonora Dondossola
Collaborators: Florian Krammer, Pedro Piedra
Department: Genomic Medicine
Phone:
Unit:

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The **primary aim** of this research study is to **determine the seroprevalence of antibodies against SARS-CoV-2, the causing agent of COVID19, among our patient population and workforce**. This study represents the initial step to determine the rate of seroposivitiy in our community. The data resulting from this study will be used to guide the development of larger ongoing study to determine the seroprevalence of SARS-CoV-2 antibodies in our population over the next 5 years.

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Primary:
- Determine the extent of SARS-CoV-2 infection in our community as determined by seropositivity.

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Severe acute respiratory disease coronavirus 2 (SARS-CoV-2) has caused a pandemic known as **Co**rona**vi**rus **D**isease 20**19** (COVID-19). Patient's symptoms range from no detectable signs of infection, to fever, cough, acute respiratory distress syndrome and pneumonia that can lead to death in 1.4% of cases[2] across the general population and up to 8-20% in more vulnerable populations (i.e. >70 years of age)[3]. Several molecular assays are currently available to identify positive patients by directly detecting viral RNA in respiratory samples through real time reverse transcriptase-PCR. However, these assays have to be performed during a narrow window following infection otherwise the virus cannot longer be detected. Irrespective of the presence of symptoms, the infection leads to the generation of antibodies that can be detected in blood for months or longer following infection offering an opportunity to determine the magnitude of cases via serologic testing. Although detecting anitbodies against SARS-CoV-2 is indicative of prior infection, it has diagnostic limitattions for the acute stages of the disease as antibodies are generated 7-28 days after infection[1]. Thus, testing of asymptomatic people has been challenging due to resource management and prioritization of symptomatic people, leaving many undiagnosed.

A serologic test for COVID19 detects the presence and measures the amount of antibodies in the blood elicited after the infection, irrespective of the absence of clinical symptoms (which have been reported as high as 88%[4]) and would complement PCR-based results to calculate the proportion of infected individuals among our community of patients and our workforce. Identifying those who have overcome the infection have great implications in our understanding of how immunity develops in cancer patients. Although limited information is

available for SARS-CoV-2, protective immunity has been shown in primates after re-challenge to CoV2, with no evidence of viral loads and no viral replication in all primary tissue compartments at five days post-reinfection[5], and in limited number of cases seroconversion (IgG and IgM) in SARS-CoV-2 has been shown to occur by 7 days in 50% of patients and 14 days in all of them, as reported[1] (Fig. 2([1])). Similar results were confirmed by others
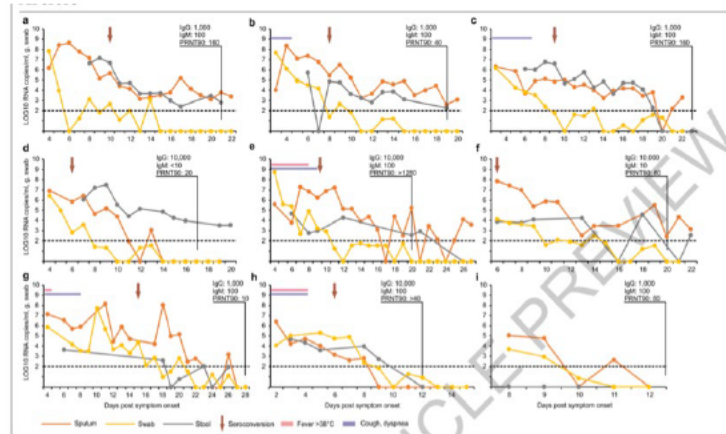
Figure 1. Adapted from Wölfer et al.[1] Viral load kinetics, seroconverstion, and clinical observations in individual cases. Panels A to I correspond to 9 different cases studies. Dotted line, limit of quantification were performed in ducplicate and the data

([6]) and evidence continues to grow. A more comprehensive study (n=175) detected neutralizing antibodies in >95% of patients positive to CoV2 using a neutralization assay (Fig. 2)[7]

There are many formats in which serologic tests can be carried out but in this case an enzyme-linked immunosorbent assay (ELISA) is the most optimal option. ELISAs provide a relatively quick (24 hours turnaround time) and inexpensive readout of antigen-antibody interaction that can be
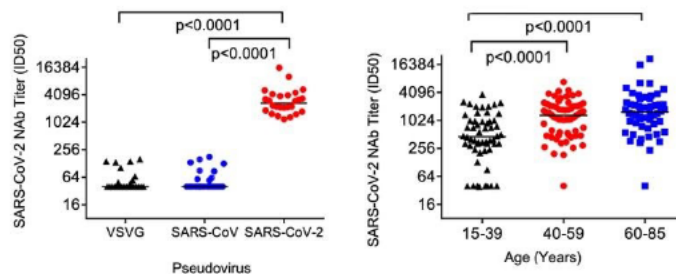
Figure 2. Adapted from X et al.

quantitative and specific for viral antigens and human antibodies. Previous work with SARS and MERS outbreaks, both caused by coronaviruses, identified an ELISA method that provided high sensitivity to detect the virus and was more efficient than other approaches. No cross-reactivity was shown to other coronaviruses (OC43, HKU1, 229E, NL63) in ELISA

assays recently developed to test SARS-CoV-2 (Fig. 3)[6,8,9]. With this novel coronavirus, initial seroprevalence in the population is assumed to be negligible due. Therefore, surveillance of antibody seropositivity in a population can allow inferences to be made about the extent of infection and about the cumulative incidence of infection in the population.

Figure 3. Adapted from X et al.

Epidemiologic studies are urgently needed to help uncover the burden of disease, in particular the rate of asymptomatic infections, and to get better estimates on illness and death. These epidemiologic studies can help identify the extent of virus spread in households, communities, and specific settings like our hospital and research buildings, which could help guide control measures. In the near future, serologic assays will be needed for evaluation of results of vaccine trials.

Hypothesis: we hypothesize that SARS-CoV-2 induces seroconversion in infected individuals stimulating the production of circulating antibodies against the virus.

3.0 Eligibility of Subjects

Inclusion: Adult subjects (≥18 years) with a laboratory confirmed diagnosis of COVID19 or under investigation for possible COVID19:
- Residual blood serum samples from laboratory confirmed COVID19 cases or from presumed cases (persons under investigation) will be obtained from the Institutional Tissue Bank (ITB) at MD Anderson Cancer Center

Exclusion: A subject will not be eligible for inclusion in this study if any of the following criteria apply:
- Samples kept in suboptimal conditions will be excluded from this study.

4.0 Research Plan and Methods

The seroepidemiological investigation for COVID19 virus among MD Anderson is intended to provide key epidemiological and serologic characteristic of the disease. The model employed will follow a repeated cross-sectional investigation in which samples will be drawn from a specific population (i.e. MD Anderson Cancer Center patients and potentially the workforce) but not necessarily from the same individuals each time. The timing of the study will depend on the our ability to return to the research laboratories.

Blood serum samples collected from individuals with confirmed laboratory results for COVID19 and others presumed to have the disease but not diagnosed (persons under investigation) will be included. These samples are collected as part of standard of care procedures and residual volumes are stored at the Institutional Tissue Bank (ITB). We will use those samples to evaluate the presence and the quantity of antibodies against SARS-CoV-2 to determine seroposivitiy rates.

The immunoassay that will be used to study the seroprevalence of antibodies against SARS-CoV-2 is being developed in collaboration with Florian Krammer, Professor of Vaccinology at the Department of Microbiology at the Icahn School of Medicine at Mount Sinai, the Principal Investigator of the Sinai-Emory Multi-Institutional Influenza Vaccine Innovation Center (SEM-CIVIC), and member of the NIH-funded Centers for Excellence in Influenza Research and Surveillance (CEIRS). Dr Krammer has shared reagents and a protocol for the development of the assay and such reagents are being shared with Dr. Pedro Piedra at Baylor College of Medicine who is leading the implementation of Dr. Krammers' protocol. Dr. Piedra is a Professor in the Department of Molecular Virology and Microbiology and his research is focused on influenza virus, respiratory syncytial virus, adenovirus viruses, human metapneumovirus, and the recently described SARS-CoV-2.

The surface glycoprotein of the virus, termed spike (S) protein, mediates attachment of the virus to human cells via its receptor binding domain (RBD) and mediates fusion of viral and cellular membranes[10]. Antibodies that bind to the spike protein, and especially to the RBD domain, can neutralize coronaviruses. This assay uses different recombinant spike protein preparations as antigen for the ELISA. It has been reported that individuals not exposed to SARS-CoV-2 are completely naïve for the spike protein and show limited reactivity in an ELISA. It is, therefore, possible to distinguish between exposed/immune and naïve people.

As described by Amanat et al[8], a two-step ELISA protocol for high throughput screening of human serum samples for antibodies binding to the spike protein of SARS-CoV-2 will be implemented. In this a two-step ELISA in which the first step includes relatively high throughput screening of samples in a single serum dilution against the RBD (which expresses very well and therefore there is typically more protein available). This is followed by a second step in which positive samples from the first step undergo a confirmatory ELISA against the full length spike protein (which is harder to purify, therefore there is usually less available). For the second step a dilution curve is performed. The throughput of this assay, if only one operator is available, screening ELISAs (760 samples/10 plates per run) and confirmatory ELISAs can be run in (140 samples/10 plates per run) in a single day. Negative controls include serum pools of serum taken before 2020. Positive controls are convalescent samples from COVID19 patients or monoclonal antibodies (mAbs) like CR30229[11,12]. If no human sera or mAbs are available, mouse mAbs, mouse sera against SARS-CoV-2, order animal sera against SARS-CoVo2 or anti-his tag antibodies (the proteins are his-tagged) can be used. However, in this case a different secondary antibody for the species from which the primary antibody is derived is needed for the positive control. The positive control exceeds an OD_{490} of the negative control plus 3 standard deviations of the negative controls up to, at least, a 1:150 dilution. ELISAs can be run with both serum and plasma.

4.1 Subjects

Patients undergoing laboratory testing for COVID19 diagnosis at MD Anderson Cancer Center from 04/1/2020 to 04/01/2021 will have their blood drawn for standard of care procedures. Residual samples collected in serum separator tubes (SST, i.e. gold or red/gray) or serum tubes (i.e. red cap) will be stored at the Institutional Tissue Bank and used for this research.

MD Anderson staff including healthcare workers and research staff presenting no symptoms for COVID19 will be enrolled to determine seroprevalence of antibodies against SARS-CoV-2. Samples will be collected in a similar way as for patients undergoing standard of care testing as explained above.

4.2 Sample Collection

Blood serum residual samples obtained from the Institutional Tissue Bank from laboratory confirmed COVID19 cases and persons under investigation (PUIs) will be assayed to detect and quantify antibodies against the viral receptor binding domain (RBD) of the spike protein of SARS-CoV-2.

Potential Harms/Adverse events:
No potential harms or adverse events are anticipated other than the very minimial risk associated with blood draws. The primary benefit of the study is indirect in that data collected will help improveour understanding of COVID19.

Safety Monitoring:
This study will be monitored by the MDACC Data and Safety Monitoring Board (DSMB).

ELISA testing should be carried out in a facility with at least biosafety level 2 (BSL-2) capacity. All personnel involved in the investigation need to be trained in infection prevention and control procedures (standard contact and droplet precautions, as determined by national or local guidelines). These procedures should include proper hand hygiene and the correct use of surgical masks, if necessary, not only to minimize their own risk of infection when in close contact with infectious material, but also to minimize the risk of spread among other participants in the investigation.

4.3 Testing

A twos-step enzyme-linked immunosorbent assay (ELISAs) is based on recombinant antigens derived from the spike protein of CoV-2, including its receptor binding domain (RBD) and full length spike (S) protein will be used. Such antigens were used for testing 59 negative human control sera preCOVID-19, including people with previous confirmed coronavirus exposure (e.g. NL63) and four samples from CoV-2 positive patients. ELISAs proved sensitive and specific and allowed for identification of positive patients using human plasma/serum as early as three days post-symptom onset (12.5 μl of plasma or serum needed/test; 2 wells, in duplicate, 6.25 μl/well). As an advantage, these assays do not require handling of infectious virus, are amenable to scaling and only few microliters of plasma/serum are needed. The assay will have sensitivity of >95% and specificity of >95% and pre-pandemic sera are used as controls to test cross reactivity to other coronavirus.

Residiual samples will be obtained from the Institutional Tissue Bank for testing. Both serum and plasma samples can be tested for the presence of antibodies against SARS-CoV-2.

5.0 Statistics and Justification of Sample Size

Sample size justification

> Commented [MOU5]: incomplete

For epidemiological studies it is important to capture a sample that is representative of the population at risk to understand the extent of the virus spread and to reduce the margin or error. Due to the fact that this is a virus newly introduced to humanity, initial seroprevalence

in the population is assumed to be minimal thus the entire population of patients and workforce is considered at risk.

Data analysis

Several epidemiological parameters will be evaluated including age-specific cumulative incidence which is defined as the proportion of individuals per age strata that are seropositive for COVID19 infection taking into account any difference the age stratification of the participants and the overall population. In addition, the proportion of asymptomatic cases over total number of confirmed cases at MD Anderson will be deterimed. Specifically, in cancer patients, we will quantify the presence of antibodies in confirmed COVID19 cases and compare it to the general population, and for patients with repeated samples, the change in levels of SARS-CoV-2 antibodies will be determined. Lastly, the case fatality ratio defined as the proporteion of individuals with fatal outcome for COVID19 infection will be established. This may require extended follow-up to determine the outcome of infection among those tested.

6.0 Procedure to Obtain Informed Consent

> **Commented [MOU6]:** Do we need IC if these are residual samples?

7.0 Data Confidentiality

Data will be available to the PI and people directly involved with the collection and analysis of data related to this project. IRB approval will be obtained for any exchange of data within and outside of MD Anderson.

Collection of Identifiers:
Identifiers (name, dates, phone number, email address and MRN) will be collected. Identifiers (other than dates) will be replaced by study numbers in the analytic file. The key linking these numbers will be retained in a locked file or stored on an encrypted server behind the firewall, by the investigator's designated personnel. Dates will be retained as a limited data set. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval.

Training of Personnel:
All MD Anderson personnel will be fully trained to maintain the patient health information confidentially. Training will be documented as required by institutional policy.

Data Storage:
The PI and research staff will attempt to minimize risk through only storing information containing subject identifiers in locked file storage, and on password-protected computers, on encrypted servers behind an institutional firewall and according to current institutional

and federal data security requirements. In addition, access to patient identifiers will be limited to the minimum number of necessary research personnel, and only to those research personnel directly involved with obtaining patient information and assigning random study identifiers. Keys containing information linking study subjects to personal identifiers will be maintained in locked storage for paper records or behind institutionally approved firewall and electronic security measures for electronic keys, and available ONLY to the PI and research personnel directly involved in creating random study identifiers. Information containing subject personal identifiers will not be removed from MD Anderson Cancer Center without IRB approval and will not be shared in publications or reports concerning this research study.

Data Sharing:
Study data will not be shared with any individuals or entities that are not involved in the study. No identifying information will be shared with outside collaborating sites or outside collaborating research staff without prior IRB approval and a data use or material transfer agreement has been implemented. Sharing of data will be done after approval of the PI and only by secure mechanisms, as approved by MD Anderson Information Security.

Final Disposition of Study Records:
These data will be used for this research study. Data that is in hard-copy form will be retained on site until the study is terminated, and may be stored indefinitely, per institutional standards, in long-term off-site storage with an MD Anderson approved, secured contract site. Electronic data will be retained indefinitely on MD Anderson servers behind the institutional firewall. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval. Study data and paper records will not be destroyed but will be retained permanently.

8.0 References

1.   Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020.
2.   Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020.
3.   Onder G, Rezza G, Brusaferro S. Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *JAMA* 2020.
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5.   Bao L, Deng W, Gao H, et al. Reinfection could not occur in SARS-CoV-2 infected rhesus macaques. *bioRxiv* 2020: 2020.03.13.990226.
6.   Okba NMA MM, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe Acute Respiratory Syndrome Coronavirus 2−Specific Antibody Responses in Coronavirus Disease 2019 Patients: Centers for Disease Control and Prevention, 2020.

7.   Wu F, Wang A, Liu M, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. *medRxiv* 2020: 2020.03.30.20047365.

8.   Amanat F, Nguyen T, Chromikova V, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *medRxiv* 2020: 2020.03.17.20037713.

9.   Guo L, Ren L, Yang S, et al. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). *Clinical Infectious Diseases* 2020.

10.   Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020; **367**(6483): 1260-3.

11.   ter Meulen J, van den Brink EN, Poon LL, et al. Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. *PLoS Med* 2006; **3**(7): e237.

12.   Tian X, Li C, Huang A, et al. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerg Microbes Infect* 2020; **9**(1): 382-5.

TITLE: Evaluating the seroprevalence of SARS-CoV02 antibodies in an asymptomatic or subclinical population at MD Anderson Cancer Center

Study Chair: Nadim Ajami
Co-Chairs: Eleonora Dondossola
Collaborators: Florian Krammer, Pedro Piedra
Department: Genomic Medicine
Phone:
Unit:

Commented [MOU1]: I don't have an academic title, can I still be PI

Commented [MOU2]: Carrie Daniel MacDougall
Paul Scheet
Alex Lazar
Roy Chemaly

Commented [MOU3]: Non-MDA - can we do this and is it smart?

Table of Contents

INTERNAL USE

PROTOCOL APPLICATION

Participant Population

Expected age range of participants:
18 and above

Will this include embryonic stem cells?
No

Does this research include MDACC employees as participants?
Yes

Total expected number of participants is 5,000 from 04/01/2020 through 04/01/2021. These are COVID19 patients or persons under investigation who undergo laboratory testing for COVID19 and where blood samples are drawn for standard of care procedures.

Specimen Type and Collection Type:
Residual blood serum samples

Total # of Specimens, Volume, Frequency:

One sample per patient or as many as available up to 5,000 patients' samples.

Commented [MOU4]: How does this sound?

List the source of specimens/data (Select all that apply):
The data of patients will be collected from the electronic medical record (Epic).

Data Profile

Types of data points that will be collected include:
Names or initials, medical record number, date of birth, gender, medications, medical comorbidities, smoking history, dates of surgery and other treatments at MD Anderson including intravenous or oral antibiotics administered and/or prescribed, dates of followup, results of imaging/pathology/labwork, date of recurrence/progression, and date of death.

Specimens/data sharing
Specimens/data will NOT be shared with any entity, person, or organization outside of MD Anderson

Confidentiality

Will there be a link to identify subjects?
YES-PHI will be collected however only made available to the PI and mid-level providers/research/clinical fellow participating in this research study

Will identifiable data be made available to anyone other than the PI?
YES-Research/clinical fellow and mid-level providers participating in this research study

Compensation
Will subjects receive compensation (e.g. water bottles, t shirts, gift cards), or reimbursements (e.g., gas cards for travel, parking vouchers, bus fare, or hotel reimbursement?)
No

Informed Consent Considerations

Please select the appropriate response(s) regarding the informed consent process for this study (you may select more than one)
Protocol staff will obtain prospective written consent

1.0 Objectives

The **primary aim** of this research study is to **determine the seroprevalence of antibodies against SARS-CoV-2, the causing agent of COVID19, among our patient population and workforce**. This study represents the initial step to determine the rate of seroposivitiy in our community. The data resulting from this study will be used to guide the development of larger ongoing study to determine the seroprevalence of SARS-CoV-2 antibodies in our population over the next 5 years.

Outcome Measures:
Primary:
- Determine the extent of SARS-CoV-2 infection in our community by means of seropositivity.

Secondary:
- Determine the cumulative incidence of infection in our patient population and workforce.
- Determine the isotype of antibodies raised against surface antigens of SARS-CoV-2

2.0 Rationale

Severe acute respiratory disease coronavirus 2 (SARS-CoV-2) has caused a pandemic known as **Co**rona**vi**rus **D**isease 20**19** (COVID-19). Patient's symptoms range from no detectable signs of infection, to fever, cough, acute respiratory distress syndrome and pneumonia that can lead to death in 1.4% of cases[1] across the general population and up to 8-20% in more vulnerable populations (i.e. >70 years of age, presence of coexisting disorders, including cancer)[2]. Several molecular assays are currently available to identify positive patients by directly detecting viral RNA in respiratory samples through real time reverse transcriptase-PCR. However, these assays have to be performed during a narrow window following infection otherwise the virus cannot longer be detected. Thus, testing of asymptomatic people has been challenging due to resource management and prioritization of symptomatic people, leaving many undiagnosed.

Irrespective of the absence of symptoms (which have been reported as high as 88%[3]), the infection leads to the generation of antibodies that can be detected in blood for months or longer following infection, offering an opportunity to determine the magnitude of cases via serologic testing. Although detecting antibodies against SARS-CoV-2 is indicative of prior exposure, it has diagnostic limitations for the acute stages of the disease as antibodies are generated 7-28 days after infection[4]. Therefore, a serologic test for COVID-19 would complement PCR-based results to calculate the proportion of infected individuals among our community of patients and our workforce. Identifying those who have overcome the infection is needed to estimate illness prevalence in the overall population and have great implications in our understanding of how immunity develops, particularly in cancer patients.

Although limited information is available for SARS-CoV-2, protective immunity has been shown in primates after re-challenge to CoV2, with no evidence of viral loads and viral replication in all primary tissue compartments at five days post-reinfection[5]. In another study, seroconversion (IgG and IgM) in SARS-CoV-2 patients has been shown to occur by 7 days in 50% of individuals and 14 days in all of them[4]

Figure 1. Adapted from Wölfer et al.[4] Viral load kinetics, seroconverstion, and clinical observations in individual cases. Panels A to I correspond to 9 different cases studies. Dotted line, limit of quantification were performed in duplicate and the data presented are means of results. Experiment obtained by two laboratories independently.

(Fig. 2([4])). Similar results were confirmed by others ([6]) and evidence continues to grow. A more comprehensive study (n=175) detected neutralizing antibodies in >95% of patients positive to CoV-2 using a neutralization assay (Fig. 2)[7]

There are many formats in which serologic tests can be carried out but in this case an enzyme-linked immunosorbent assay (ELISA) is the most optimal option. ELISAs provide a relatively quick (>24 hours turnaround time) and inexpensive readout of antigen-antibody interaction that can be

Figure 2. Adapted from Wu et al.[7] Titers of neutralizing Abs against VSV, SARS-CoV, and SARS-CoV-2 pseudovirus in 26 COVID-19 recovered patient plasma. More than 95% developed antbodies above the detection limit (50 OD).

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surveillance of antibody seropositivity in a population can allow inferences to be made about the extent of infection and about the cumulative incidence of infection in the population.

Fig. 4. Adapted from Okba et al. [6] 87%–100% of serum samples in control cohorts (blood donors, non-CoV infection, HCoV) were seropositive for endemic HCoVs (HCoV-HKU1, HCoV-OC43, HCoV-NL63, and HCoV-229E), as determined by the S1 protein microarray. No antibody cross-reactivity was shown with SARS-CoV-2.

Based on this evidence, epidemiologic studies are urgently needed to help uncover the burden of disease, in particular the rate of asymptomatic infections, and to get better estimates on illness and death. These studies can help identify the extent of virus spread in households, communities, and specific settings like our hospital and research buildings, which could help guide control measures. In the near future, serologic assays will be needed for evaluation of results of vaccine trials.

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The immunoassay that will be used to study the seroprevalence of antibodies against SARS-CoV-2 is being developed in collaboration with Florian Krammer, Professor of Vaccinology at the Department of Microbiology at the Icahn School of Medicine at Mount Sinai, the Principal Investigator of the Sinai-Emory Multi-Institutional Influenza Vaccine Innovation Center (SEM-CIVIC), and member of the NIH-funded Centers for Excellence in Influenza Research and Surveillance (CEIRS).
The IgG ELISA developed by Dr. Krammer received Emergency Use Approval by the FDA on April 16 (https://www.fda.gov/media/137032/download). He has shared reagents and a protocol for the development of the assay with the Chair and Co-Chair of this study and such reagents are being further shared with Dr. Pedro Piedra at Baylor College of Medicine who is leading the local implementation of Dr. Krammers' protocol. Dr. Piedra is a Professor in the Department of Molecular Virology and Microbiology and his research is focused on influenza virus, respiratory syncytial virus, adenovirus viruses, human metapneumovirus, and the recently described SARS-CoV-2.

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As described by Amanat et al[8], a two-step ELISA protocol for high throughput screening of human serum samples for antibodies binding to the spike protein of SARS-CoV-2 will be implemented. In this a two-step ELISA. The first step includes relatively high throughput screening of samples in a single serum dilution against recombinant RBD produced in mammalian cells (which expresses very well and therefore leads to a higher protein yield). This is followed by a second step in which positive samples from the first step undergo a confirmatory ELISA against the full length spike protein (which is harder to purify, therefore there is usually less available). For the second step a dilution curve is performed. The throughput of this assay, if only one operator is available, allows to perform screening ELISAs (760 samples/10 plates per run) and confirmatory ELISAs in (140 samples/10 plates per run) in a single day. Negative controls include serum pools of serum taken before 2020. Positive controls are convalescent samples from COVID19 patients or monoclonal antibodies (mAbs) like CR30229[11,12]. If no human sera or mAbs are available, mouse mAbs, mouse sera against SARS-CoV-2, order animal sera against SARS-CoVo2 or anti-his tag antibodies (the recombinant proteins used as antigens are his-tagged) can be used. However, in this case a different secondary antibody for the species from which the primary antibody is derived is needed for the positive control. The positive control exceeds an OD_{490} of the negative control plus 3 standard deviations of the negative controls up to, at least, a 1:150 dilution. ELISAs can be run with both serum and plasma.

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MD Anderson staff including healthcare workers and research staff presenting no symptoms for COVID19 will be enrolled under the APOLLO protocol to determine seroprevalence of antibodies against SARS-CoV-2. Samples will be collected in a similar way as for patients undergoing standard of care testing as explained above.

Commented [MOU5]: Need to verify that blood samples are collected in red or red/grey cap tubes

4.2 Sample Collection

Residual samples obtained from the Institutional Tissue Bank from laboratory confirmed COVID19 cases and persons under investigation (PUIs) and prospectilvey collected samples from MD Anderson staff will be assayed to detect and quantify antibodies against the viral receptor binding domain (RBD) of the spike protein of SARS-CoV-2. Both serum and plasma samples can be tested for the presence of antibodies against SARS-CoV-2.

Potential Harms/Adverse events:
No potential harms or adverse events are anticipated other than the very minimal risk associated with blood draws. The primary benefit of the study is indirect in that data collected will help improve our understanding of COVID19.

Safety Monitoring:
This study will be monitored by the MDACC Data and Safety Monitoring Board (DSMB).

ELISA testing should be carried out in a facility with at least biosafety level 2 (BSL-2) capacity. All personnel involved in the investigation need to be trained in infection prevention and control procedures (standard contact and droplet precautions, as determined by national or local guidelines). These procedures should include proper hand hygiene and the correct use of surgical masks, if necessary, not only to minimize their own risk of infection when in close contact with infectious material, but also to minimize the risk of spread among other participants in the investigation.

4.3 Testing

A two-step enzyme-linked immunosorbent assay (ELISAs) based on recombinant antigens derived from the spike protein of CoV-2, including its receptor binding domain (RBD) and full length spike (S) protein will be used. Such antigens were used for testing 59 negative human control sera preCOVID-19, including people with previous confirmed coronavirus exposure (e.g. NL63) and four samples from CoV-2 positive patients. ELISAs proved sensitive and specific and allowed for identification of positive patients using human plasma/serum as early as three days post-symptom onset (12.5 µl of plasma or serum needed/test; 2 wells, in duplicate, 6.25 µl/well). As an advantage, these assays do not require handling of infectious virus, are amenable to scaling and only few microliters of plasma/serum are needed. The assay will have sensitivity of >95% and specificity of >95% and pre-pandemic sera are used as controls to test cross reactivity to other coronavirus.
As mentioned, this assay got Emergency Use Approval by the FDA.

Detailed Protocol (Adapted from Stadlbauer et al, A detailed protocol for a serological assay to detect SARS-CoV-2 1 seroconversion in humans: antigen production and test setup).

A - RBD Screening ELISA

1. Coating ELISA plates (day 1)
● Thaw the required number of vials of antigen (SARS-CoV-2 **RBD** protein) to coat 96-well microtiter ELISA plates at a concentration of 2 µg/ml. Once thawed, mix by gently vortexing vial before diluting in 1X PBS.
● Prepare approximately 5 mL for each plate to be coated.
● Coat plates with 50 µl of diluted protein per well using a multichannel pipette and a reservoir. Lightly tap plates against surface to ensure protein is evenly coating the bottom of every well.

● Incubate at 4°C overnight. Plates can be stored at 4°C for up to 1 week.
● Always keep a cover plate on top of coated plates during all steps of the protocol!

2. Heat inactivation of samples (day 1, this is a safety precaution)
● Set the water bath to 56°C. Once temperature is reached, place the serum/plasma samples in and start the timer for 1h immediately.
● Remove samples when the timer goes off. Do not leave samples at 56°C for longer than 1h. Store at 4°C overnight or until use.

3. Block ELISA plate (day 2)
● Calculate to prepare at least 30 ml of blocking solution per plate.
● Blocking solution consists of PBS-T + 3% milk powder (weight/volume).
● Using an automated plate washer, wash coated ELISA plates 3x with PBS-T.
● Add 200 µl blocking solution to all wells of the plates, starting the timer for 1h (do not exceed 4h) after completing the first plate. Place plates in a 20°C (RT) incubator.
Note: This step (and wherever a plate washer is needed below) can also be performed by washing plates with a multichannel pipette by hand if no plate washer is available.

4. Pre-diluting samples (day 2)
● In a biological safety cabinet, set up sterile Eppendorf tubes to pre-dilute serum samples 1:5.
● Add 40 µl of sterile 1X PBS to all tubes.
● Gently vortex serum sample to mix and add 10 µl to the Eppendorf tube, vortexing once more. Do this for all remaining samples including the positive and negative controls. *Volume not needed in this part A will be stored and used for part B.*

5. Dilution plate set-up (day 2)
● Calculate and prepare at least 30 ml of PBS-T + 1% mi k powder (weight/volume).
● Prepare 1 dilution plate (separate flat bottomed cell culture plate) per antigen coated plate prepared.
● Add 180 µl of PBS-T containing 1% mi k to all wells of the dilution plate (including blank wells)
● Leaving Columns 1 and 12 as blanks, add 20 µl of sample (or control) into the designated well. This results in a final serum dilution of 1:50.
● Continue until all samples and controls have been added to designated wells. See reference plate layout below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	(+) Ctrl.	(-) Ctrl.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Blank
B	Blank	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	...	...	...			Blank
C	Blank											Blank
D	Blank											Blank
E	Blank											Blank
F	Blank											Blank
G	Blank											Blank
H	Blank		...	...	...	Sample 73	Sample 74	Sample 75	Sample 76	(+) Ctrl.	(-) Ctrl.	Blank

6. Transfer serum dilution (day 2)
● After blocking incubation, remove plates from the room temperature incubator and throw off the blocking solution. Tap the plates dry on a kimwipe.
● Using a multichannel pipette, pipette up and down 4-6 times in the first row of dilution plate to mix.
● Transfer 100 µl to the corresponding rows in the ELISA plate. Change tips and continue to transfer second row to the ELISA plate.
● Start the timer for 2h as soon as all the rows have been transferred to the first ELISA 374 plate. (Do not exceed 4h)
● Place plates in a 20°C (RT) incubator.

7. Secondary Antibody (day 2)
● After 2h, wash the plates 3x with PBS-T using the automated plate washer.
● Dilute anti-human IgG (Fab specific) HRP labeled secondary antibody 1:3000 in PBS-T containing 1% milk. Prepare at least 5 ml per plate.
● Add 50 µl to all wells of the plate using a multichannel pipette. Be sure to avoid touching the tips of the pipette to the walls of the well to avoid carry over and high background signals.
● Start the timer for 1h (stay in a range of 50min to 65min) as soon as the secondary antibody has been added to the first plate. Place plates in a 20°C (RT) incubator.

8. Plate development and reading (day 2)
● After 1h, wash plates 3x with PBS-T using an automated plate washer.
● Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1 gold + 1 silver tablet) dissolved in 20 ml WFI can be used for 2 plates.
● Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until ready to start adding to the plates (needs to be prepared fresh right before use).
● Add 100 µl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has been added to the first row on the first plate. Do not exceed 10 minutes of developing before stopping the reaction.
● To stop the reaction after exactly 10 minutes, add 50 µl of 3M HCl to all wells.
● Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after adding HCl) and record data.
● Samples that exceed certain OD_{490} cutoff value (proposed cutoff: OD_{490} = 0.15-0.2 or mean of negative controls plus 3 times the standard deviation of the negative controls) are assigned presumptive positive and will be tested in confirmatory ELISA using full-length spike protein.
● OD_{490} cutoff has to be experimentally determined and depends on assay background 404 and noise.

B - Spike confirmatory ELISA

1. Coating ELISA plates (day 1)
● Thaw the required number of vials of antigen (SARS-CoV-2 **Spike** protein) to coat 96-well microtiter ELISA plates at a concentration of 2 µg/ml. Once thawed, mix by gently vortexing vial before diluting in 1X PBS.
● Prepare approximately 5 mL for each plate to be coated.
● Coat plates with 50 µl of diluted protein per well using a multichannel pipette and a reservoir. Lightly tap plates against surface to ensure protein is evenly coating the bottom of every well.
● Incubate at 4°C overnight. Plates can likely be stored in 4°C for up to 1 week but this needs to be validate locally to ascertain that it does not change rhe results.

2. Block ELISA plate (day 2)
● Calculate to prepare at least 30 ml of blocking solution per plate.
● Blocking solution consists of PBS-T + 3% milk powder (weight/volume).
● Using an automated plate washer, wash coated ELISA plates 3x with PBS-T.
● Add 200 µl blocking solution to all wells of the plates, starting the timer for 1 h (do not exceed 4h) after completing the first plate. Place plates in a 20°C (RT) incubator.

3. Pre-diluting samples (day 2)
● Retrieve 1:5 pre-diluted samples from Part A to be tested and confirmed (samples that are above certain threshold in RBD screening ELISA based on a set OD_{490} value –see end of **A**).

4. Serial dilution (day 2)
● Calculate and prepare at least 20 ml of PBS-T + 1% mi k powder (weight/volume) per plate.
● After blocking incubation, remove plates from the room temperature incubator and throw off the blocking solution. Tap the plates dry on a kimwipe.
● Using a multichannel pipette, add 120 µl of PBS-T containing 1% milk to all wells of the plate.
● Leaving Columns 1 and 12 as BLANKS, add an extra 51 µl only to Columns 2 and 7 (=sample wells).
● Add 9 µl of 1:5 pre-diluted sample (final dilution 1:100 on the plate) to the first well in Column 2 and continue to add samples to all 8 wells. In Column 7, add samples to wells 1 through 6. Transfer positive and negative control into wells 7 and 8, respectively. See reference plate layout below.
● With the multichannel pipette, pipette up and down 4-6 times in Column 2 to mix. Discard these tips. With new tips, transfer 60 µl (3-fold dilution) from Column 2 to Column 3 and pipette up and down once 4-6 times to mix. Repeat this until Column 6; 450 discard 60 µl before Column 7.
● Taking fresh tips mix Column 7 by pipetting. Repeat the same process of transferring, mixing, and discarding tips from Columns 7-11. Once Column 11 is reached, discard 60 µl.
● Start timer for 2h (do not exceed 4h) once the first ELISA plate has been serially diluted.
● Place plates in a 20°C (RT) incubator.

5. Secondary Antibody (day 2)
● After 2h, wash the plates 3x with PBS-T using the automated plate washer.
● Dilute anti-human IgG (Fab specific) HRP labeled secondary antibody 1:3000 in PBS-T containing 1% milk. Prepare at least 5 ml per plate.
● Add 50 µl to all wells of the plate using a multichannel pipette. Be sure to avoid 466 touching the tips of the pipette to the walls of the well.
● Start the timer for 1 h (50 – 65 min) as soon as the secondary antibody has been 468 added to the first plate. Place plates in a 20°C (RT) incubator.

6. Plate development and reading (day 2)
● After 1h, wash plates 3x with PBS-T using an automated plate washer.
● Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1 gold + 1 silver tablet) dissolved in 20 mL WFI can be used for 2 plates.
● Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until 475 ready to start adding to the plates.
● Add 100 µl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has been added to the first row of the first plate. Do not exceed 10 minutes of developing before stopping the reaction.
● To stop the reaction after exactly 10 minutes, add 50 µl of 3M HCl to all wells.
● Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after adding HCl) and record data.
● True positive samples will have a signal higher than the negative control plus 3 standard deviations of the negative controls in at least two consecutive dilutions.

5.0 Statistics and Justification of Sample Size

Sample size justification

Commented [MOU6]: incomplete

For epidemiological studies it is important to capture a sample that is representative of the population at risk to understand the extent of the virus spread and to reduce the margin or

error. Due to the fact that this is a virus newly introduced to humanity, initial seroprevalence in the population is assumed to be minimal thus the entire population of patients and workforce is considered at risk.

Data analysis

Several epidemiological parameters will be evaluated including age-specific cumulative incidence which is defined as the proportion of individuals per age strata that are seropositive for COVID19 infection taking into account any difference the age stratification of the participants and the overall population. In addition, the proportion of asymptomatic cases over total number of confirmed cases at MD Anderson will be determined. Specifically, in cancer patients, we will quantify the presence of antibodies in confirmed COVID19 cases and compare it to the general population, and for patients with repeated samples, the change in levels of SARS-CoV-2 antibodies will be determined. Lastly, the case fatality ratio defined as the proporteion of individuals with fatal outcome for COVID19 infection will be established. This may require extended follow-up to determine the outcome of infection among those tested.

6.0 Procedure to Obtain Informed Consent

> **Commented [MOU7]:** Do we need IC if we are using residual samples and others collected under APOLLO?

7.0 Data Confidentiality

Data will be available to the PI and people directly involved with the collection and analysis of data related to this project. IRB approval will be obtained for any exchange of data within and outside of MD Anderson.

<u>Collection of Identifiers:</u>
Identifiers (name, dates, phone number, email address and MRN) will be collected. Identifiers (other than dates) will be replaced by study numbers in the analytic file. The key linking these numbers will be retained in a locked file or stored on an encrypted server behind the firewall, by the investigator's designated personnel. Dates will be retained as a limited data set. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval.

<u>Training of Personnel:</u>
All MD Anderson personnel will be fully trained to maintain the patient health information confidentially. Training will be documented as required by institutional policy.

<u>Data Storage:</u>
The PI and research staff will attempt to minimize risk through only storing information containing subject identifiers in locked file storage, and on password-protected computers,

on encrypted servers behind an institutional firewall and according to current institutional and federal data security requirements. In addition, access to patient identifiers will be limited to the minimum number of necessary research personnel, and only to those research personnel directly involved with obtaining patient information and assigning random study identifiers. Keys containing information linking study subjects to personal identifiers will be maintained in locked storage for paper records or behind institutionally approved firewall and electronic security measures for electronic keys, and available ONLY to the PI and research personnel directly involved in creating random study identifiers. Information containing subject personal identifiers will not be removed from MD Anderson Cancer Center without IRB approval and will not be shared in publications or reports concerning this research study.

Data Sharing:
Study data will not be shared with any individuals or entities that are not involved in the study. No identifying information will be shared with outside collaborating sites or outside collaborating research staff without prior IRB approval and a data use or material transfer agreement has been implemented. Sharing of data will be done after approval of the PI and only by secure mechanisms, as approved by MD Anderson Information Security.

Final Disposition of Study Records:
These data will be used for this research study. Data that is in hard-copy form will be retained on site until the study is terminated, and may be stored indefinitely, per institutional standards, in long-term off-site storage with an MD Anderson approved, secured contract site. Electronic data will be retained indefinitely on MD Anderson servers behind the institutional firewall. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval. Study data and paper records will not be destroyed but will be retained permanently.

8.0 References

1.   Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020.
2.   Onder G, Rezza G, Brusaferro S. Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *JAMA* 2020.
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TITLE: Evaluating the seroprevalence of SARS-CoV02 antibodies in an asymptomatic or subclinical population at MD Anderson Cancer Center

Study Chair: Nadim Ajami
Co-Chairs: Eleonora Dondossola
Internal Collaborators: Roy Chemaly, Padmanee Sharma, Jennifer Wargo, Alex Lazar
External Collaborators: Florian Krammer, Pedro Piedra
Department: Genomic Medicine
Phone:
Unit:

Commented [MOU1]: I don't have an academic title, can I still be PI

Commented [MOU2]: Carrie Daniel MacDougall
Paul Scheet
Alex Lazar
Roy Chemaly

Commented [MOU3]: Non-MDA - can we do this and is it smart?

Table of Contents

==INTERNAL USE==

PROTOCOL APPLICATION

Participant Population

Expected age range of participants:
18 and above

Will this include embryonic stem cells?
No

Does this research include MDACC employees as participants?
Yes

Total expected number of participants is 5,000 from 04/01/2020 through 04/01/2021. These are COVID19 patients or persons under investigation who undergo laboratory testing for COVID19 and where blood samples are drawn for standard of care procedures.

Specimen Type and Collection Type:
Residual blood serum samples and prospectively collected samples

Total # of Specimens, Volume, Frequency:

One sample per patient or as many as available up to 5,000 patients' samples.

> **Commented [MOU4]:** How does this sound?

For residual blood serum samples, a minimum of xx mL will be required. For prospectively collected samples, 5-10 mL of blood will be drawn per institutional standards.

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> **Commented [MOU5]:** Nadim – please work with Liz Burton on filling in these details. Check with Roy Chemaly as well

List the source of specimens/data (Select all that apply):
Residual blood serum samples will be obtained from the Department of Pathology and Laboratory Medicine.
Prospectively collected samples will be drawn using an institutional Z-code under the APOLLO protocol and processed and banked through the institutional tissue bank (ITB).
The data ofRelevant metadata from patients will be collected from the electronic medical record (Epic).

Data Profile

Types of data points that will be collected include:
Names or initials, medical record number, date of birth, gender, medications, medical comorbidities, smoking history, dates of surgery and other treatments at MD Anderson including intravenous or oral antibiotics administered and/or prescribed, dates of followup, results of imaging/pathology/labwork, date of recurrence/progression, and date of death.

Specimens/data sharing
Specimens/data will ~~NOT~~ be shared with ~~any entity~~entities, person<u>s</u>, or organization<u>s</u> outside of MD Anderson <u>only with proper approval and material transfer agreement (MTA)</u>

Confidentiality

Will there be a link to identify subjects?
YES-PHI will be collected however only made available to the PI and mid-level providers/research/clinical fellow participating in this research study

Will identifiable data be made available to anyone other than the PI?
YES-Research/clinical fellow and mid-level providers participating in this research study

Compensation
Will subjects receive compensation (e.g. water bottles, t shirts, gift cards), or reimbursements (e.g., gas cards for travel, parking vouchers, bus fare, or hotel reimbursement?)
No

Informed Consent Considerations

Please select the appropriate response(s) regarding the informed consent process for this study (you may select more than one)
Protocol staff will obtain prospective written consent

1.0 Objectives

The **primary aim** of this research study is to **determine the seroprevalence of antibodies against SARS-CoV-2, the causing agent of COVID19, among our patient population and workforce**. This study represents the initial step to determine the rate of seroposivitiy in our community. The data resulting from this study will be used to guide the development of larger ongoing study to determine the seroprevalence of SARS-CoV-2 antibodies in our population over the next 5 years.

Outcome Measures:
Primary:
- Determine the extent of SARS-CoV-2 infection in our community by means of seropositivity.

Secondary:
- Determine the cumulative incidence of infection in our patient population and workforce.
- Determine the isotype of antibodies raised against surface antigens of SARS-CoV-2

2.0 Rationale

Severe acute respiratory disease coronavirus 2 (SARS-CoV-2) has caused a pandemic known as **Co**rona**vi**rus **D**isease 20**19** (COVID-19). Patient's symptoms range from no detectable signs of infection, to fever, cough, acute respiratory distress syndrome and pneumonia that can lead to death in 1.4% of cases[1] across the general population and up to 8-20% in more vulnerable populations (i.e. >70 years of age, presence of coexisting disorders, including cancer)[2]. Several molecular assays are currently available to identify positive patients by directly detecting viral RNA in respiratory samples through real time reverse transcriptase-PCR. However, these assays have to be performed during a narrow window following infection otherwise the virus cannot longer be detected. Thus, testing of asymptomatic people has been challenging due to resource management and prioritization of symptomatic people, leaving many undiagnosed.

Irrespective of the absence of symptoms (which have been reported as high as 88%[3]), the infection leads to the generation of antibodies that can be detected in blood for months or longer following infection, offering an opportunity to determine the magnitude of cases via serologic testing. Although detecting antibodies against SARS-CoV-2 is indicative of prior exposure, it has diagnostic limitations for the acute stages of the disease as antibodies are generated 7-28 days after infection[4]. Therefore, a serologic test for COVID-19 would complement PCR-based results to calculate the proportion of infected individuals among our community of patients and our workforce. Identifying those who have overcome the infection is needed to estimate illness prevalence in the overall population and have great implications in our understanding of how immunity develops, particularly in cancer patients.

Although limited information is available for SARS-CoV-2, protective immunity has been shown in primates after re-challenge to CoV2, with no evidence of viral loads and viral replication in all primary tissue compartments at five days post-reinfection[5]. In another study, seroconversion (IgG and IgM) in SARS-CoV-2 patients has been shown to occur by 7 days in 50% of individuals and 14 days in all of them[4]

Figure 1. Adapted from Wölfer et al.[4] Viral load kinetics, seroconverstion, and clinical observations in individual cases. Panels A to I correspond to 9 different cases studies. Dotted line, limit of quantification were performed in duplicate and the data presented are means of results. Experiment obtained by two laboratories independently.

(Fig. 2([4])). Similar results were confirmed by others ([6]) and evidence continues to grow. A more comprehensive study (n=175) detected neutralizing antibodies in >95% of patients positive to CoV-2 using a neutralization assay (Fig. 2)[7]

There are many formats in which serologic tests can be carried out but in this case an enzyme-linked immunosorbent assay (ELISA) is the most optimal option. ELISAs provide a relatively quick (>24 hours turnaround time) and inexpensive readout of antigen-antibody interaction that can be

Figure 2. Adapted from Wu et al.[7] Titers of neutralizing Abs against VSV, SARS-CoV, and SARS-CoV-2 pseudovirus in 26 COVID-19 recovered patient plasma. More than 95% developed antbodies above the detection limit (50 OD).

quantitative and specific for viral antigens and human antibodies. Previous work with SARS and MERS outbreaks, both caused by coronaviruses, identified an ELISA method that provided high sensitivity to detect the virus and was more efficient than other approaches. No cross-reactivity was shown to other coronaviruses (OC43, HKU1, 229E, NL63) in ELISA assays recently developed to test SARS-CoV-2 (Fig. 3)[6,8,9]. With this novel coronavirus, initial seroprevalence in the population is assumed to be negligible due. Therefore,

surveillance of antibody seropositivity in a population can allow inferences to be made about the extent of infection and about the cumulative incidence of infection in the population.

Fig. 4. Adapted from Okba et al. [6] 87%–100% of serum samples in control cohorts (blood donors, non-CoV infection, HCoV) were seropositive for endemic HCoVs (HCoV-HKU1, HCoV-OC43, HCoV-NL63, and HCoV-229E), as determined by the S1 protein microarray. No antibody cross-reactivity was shown with SARS-CoV-2.

Based on this evidence, epidemiologic studies are urgently needed to help uncover the burden of disease, in particular the rate of asymptomatic infections, and to get better estimates on illness and death. These studies can help identify the extent of virus spread in households, communities, and specific settings like our hospital and research buildings, which could help guide control measures. In the near future, serologic assays will be needed for evaluation of results of vaccine trials.

Hypothesis: we hypothesize that SARS-CoV-2 induces seroconversion in infected individuals stimulating the production of circulating antibodies against the virus.

3.0 Eligibility of Subjects

Inclusion: Adult subjects (≥18 years) with a laboratory confirmed diagnosis of COVID19 or under investigation for possible COVID19:
- Residual blood serum samples from laboratory confirmed COVID19 cases or from presumed cases (persons under investigation) will be obtained from the Institutional Tissue Bank (ITB) at MD Anderson Cancer Center

Exclusion: A subject will not be eligible for inclusion in this study if any of the following criteria apply:
- Samples kept in suboptimal conditions will be excluded from this study.

4.0 Research Plan and Methods

The seroepidemiological investigation for COVID19 virus among MD Anderson is intended to provide key epidemiological and serologic characteristic of the disease. The model employed will follow a repeated cross-sectional investigation in which samples will be drawn from a specific population (i.e. MD Anderson Cancer Center patients and potentially the workforce) but not necessarily from the same individuals each time. The timing of the study will depend on our ability to return to the research laboratories.

Blood serum samples collected from individuals with confirmed laboratory results for COVID19 and others presumed to have the disease but not diagnosed (persons under investigation) will be included. These samples are collected as part of standard of care procedures and residual volumes are stored at the Institutional Tissue Bank (ITB). We will use those samples to evaluate the presence and the quantity of antibodies against SARS-CoV-2 to determine seroposivitiy rates.

The immunoassay that will be used to study the seroprevalence of antibodies against SARS-CoV-2 is being developed in collaboration with Florian Krammer, Professor of Vaccinology at the Department of Microbiology at the Icahn School of Medicine at Mount Sinai, the Principal Investigator of the Sinai-Emory Multi-Institutional Influenza Vaccine Innovation Center (SEM-CIVIC), and member of the NIH-funded Centers for Excellence in Influenza Research and Surveillance (CEIRS).

The IgG ELISA developed by Dr. Krammer received Emergency Use Approval by the FDA on April 16 (https://www.fda.gov/media/137032/download). He has shared reagents and a protocol for the development of the assay with the Chair and Co-Chair of this study and such reagents are being further shared with Dr. Pedro Piedra at Baylor College of Medicine who is leading the local implementation of Dr. Krammers' protocol. Dr. Piedra is a Professor in the Department of Molecular Virology and Microbiology and his research is focused on influenza virus, respiratory syncytial virus, adenovirus viruses, human metapneumovirus, and the recently described SARS-CoV-2.

The surface glycoprotein of the virus, termed spike (S) protein, mediates attachment of the virus to human cells via its receptor binding domain (RBD) and subsequent fusion of viral and cellular membranes[10]. Antibodies that bind to the spike protein, and especially to the RBD domain, can neutralize coronaviruses. This assay uses different recombinant spike protein preparations as antigen for the ELISA. It has been reported that individuals not exposed to SARS-CoV-2 are completely naïve for the spike protein and show no reactivity in an ELISA[6,8,9] test. It is, therefore, possible to distinguish between exposed/immune and naïve people.

As described by Amanat et al[8], a two-step ELISA protocol for high throughput screening of human serum samples for antibodies binding to the spike protein of SARS-CoV-2 will be implemented. In this a two-step ELISA. The first step includes relatively high throughput screening of samples in a single serum dilution against recombinant RBD produced in mammalian cells (which expresses very well and therefore leads to a higher protein yield). This is followed by a second step in which positive samples from the first step undergo a confirmatory ELISA against the full length spike protein (which is harder to purify, therefore there is usually less available). For the second step a dilution curve is performed. The throughput of this assay, if only one operator is available, allows to perform screening ELISAs (760 samples/10 plates per run) and confirmatory ELISAs in (140 samples/10 plates per run) in a single day. Negative controls include serum pools of serum taken before 2020. Positive controls are convalescent samples from COVID19 patients or monoclonal antibodies (mAbs) like CR30229[11,12]. If no human sera or mAbs are available, mouse mAbs, mouse sera against SARS-CoV-2, order animal sera against SARS-CoVo2 or anti-his tag antibodies (the recombinant proteins used as antigens are his-tagged) can be used. However, in this case a different secondary antibody for the species from which the primary antibody is derived is needed for the positive control. The positive control exceeds an OD_{490} of the negative control plus 3 standard deviations of the negative controls up to, at least, a 1:150 dilution. ELISAs can be run with both serum and plasma.

4.1 Subjects

Patients undergoing laboratory testing for COVID19 diagnosis at MD Anderson Cancer Center from 04/1/2020 to 04/01/2021 will have their blood drawn for standard of care procedures. Residual samples collected in serum separator tubes (SST, i.e. gold or red/gray) or serum tubes (i.e. red cap) will be stored at the Institutional Tissue Bank and used for this research.

MD Anderson staff including healthcare workers and research staff presenting no symptoms for COVID19 will be enrolled under the APOLLO protocol to determine seroprevalence of antibodies against SARS-CoV-2. Samples will be collected in a similar way as for patients undergoing standard of care testing as explained above.

> **Commented [MOU7]:** Need to verify that blood samples are collected in red or red/grey cap tubes

4.2 Sample Collection

Residual samples obtained from the Institutional Tissue Bank from laboratory confirmed COVID19 cases and persons under investigation (PUIs) and prospectilvey collected samples from MD Anderson staff will be assayed to detect and quantify antibodies against the viral receptor binding domain (RBD) of the spike protein of SARS-CoV-2. Both serum and plasma samples can be tested for the presence of antibodies against SARS-CoV-2.

Potential Harms/Adverse events:
No potential harms or adverse events are anticipated other than the very minimal risk associated with blood draws. The primary benefit of the study is indirect in that data collected will help improve our understanding of COVID19.

Safety Monitoring:
This study will be monitored by the MDACC Data and Safety Monitoring Board (DSMB).

ELISA testing should be carried out in a facility with at least biosafety level 2 (BSL-2) capacity. All personnel involved in the investigation need to be trained in infection prevention and control procedures (standard contact and droplet precautions, as determined by national or local guidelines). These procedures should include proper hand hygiene and the correct use of surgical masks, if necessary, not only to minimize their own risk of infection when in close contact with infectious material, but also to minimize the risk of spread among other participants in the investigation.

4.3 Testing

A two-step enzyme-linked immunosorbent assay (ELISAs) based on recombinant antigens derived from the spike protein of CoV-2, including its receptor binding domain (RBD) and full length spike (S) protein will be used. Such antigens were used for testing 59 negative human control sera preCOVID-19, including people with previous confirmed coronavirus exposure (e.g. NL63) and four samples from CoV-2 positive patients. ELISAs proved sensitive and specific and allowed for identification of positive patients using human plasma/serum as early as three days post-symptom onset (12.5 µl of plasma or serum needed/test; 2 wells, in duplicate, 6.25 µl/well). As an advantage, these assays do not require handling of infectious virus, are amenable to scaling and only few microliters of plasma/serum are needed. The assay will have sensitivity of >95% and specificity of >95% and pre-pandemic sera are used as controls to test cross reactivity to other coronavirus.
As mentioned, this assay got Emergency Use Approval by the FDA.

Detailed Protocol (Adapted from Stadlbauer et al, A detailed protocol for a serological assay to detect SARS-CoV-2 1 seroconversion in humans: antigen production and test setup).

A - RBD Screening ELISA

1. Coating ELISA plates (day 1)
● Thaw the required number of vials of antigen (SARS-CoV-2 **RBD** protein) to coat 96-well microtiter ELISA plates at a concentration of 2 µg/ml. Once thawed, mix by gently vortexing vial before diluting in 1X PBS.
● Prepare approximately 5 mL for each plate to be coated.
● Coat plates with 50 µl of diluted protein per well using a multichannel pipette and a reservoir. Lightly tap plates against surface to ensure protein is evenly coating the bottom of every well.

● Incubate at 4°C overnight. Plates can be stored at 4°C for up to 1 week.
● Always keep a cover plate on top of coated plates during all steps of the protocol!

2. Heat inactivation of samples (day 1, this is a safety precaution)
● Set the water bath to 56°C. Once temperature is reached, place the serum/plasma samples in and start the timer for 1h immediately.
● Remove samples when the timer goes off. Do not leave samples at 56°C for longer than 1h. Store at 4°C overnight or until use.

3. Block ELISA plate (day 2)
● Calculate to prepare at least 30 ml of blocking solution per plate.
● Blocking solution consists of PBS-T + 3% milk powder (weight/volume).
● Using an automated plate washer, wash coated ELISA plates 3x with PBS-T.
● Add 200 µl blocking solution to all wells of the plates, starting the timer for 1h (do not exceed 4h) after completing the first plate. Place plates in a 20°C (RT) incubator.
Note: This step (and wherever a plate washer is needed below) can also be performed by washing plates with a multichannel pipette by hand if no plate washer is available.

4. Pre-diluting samples (day 2)
● In a biological safety cabinet, set up sterile Eppendorf tubes to pre-dilute serum samples 1:5.
● Add 40 µl of sterile 1X PBS to all tubes.
● Gently vortex serum sample to mix and add 10 µl to the Eppendorf tube, vortexing once more. Do this for all remaining samples including the positive and negative controls. *Volume not needed in this part A will be stored and used for part B.*

5. Dilution plate set-up (day 2)
● Calculate and prepare at least 30 ml of PBS-T + 1% mi k powder (weight/volume).
● Prepare 1 dilution plate (separate flat bottomed cell culture plate) per antigen coated plate prepared.
● Add 180 µl of PBS-T containing 1% mi k to all wells of the dilution plate (including blank wells)
● Leaving Columns 1 and 12 as blanks, add 20 µl of sample (or control) into the designated well. This results in a final serum dilution of 1:50.
● Continue until all samples and controls have been added to designated wells. See reference plate layout below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	(+) Ctrl.	(-) Ctrl.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Blank
B	Blank	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	…	…	…			Blank
C	Blank											Blank
D	Blank											Blank
E	Blank											Blank
F	Blank											Blank
G	Blank											Blank
H	Blank		…	…	…	Sample 73	Sample 74	Sample 75	Sample 76	(+) Ctrl.	(-) Ctrl.	Blank

6. Transfer serum dilution (day 2)
● After blocking incubation, remove plates from the room temperature incubator and throw off the blocking solution. Tap the plates dry on a kimwipe.
● Using a multichannel pipette, pipette up and down 4-6 times in the first row of dilution plate to mix.
● Transfer 100 μl to the corresponding rows in the ELISA plate. Change tips and continue to transfer second row to the ELISA plate.
● Start the timer for 2h as soon as all the rows have been transferred to the first ELISA 374 plate. (Do not exceed 4h)
● Place plates in a 20°C (RT) incubator.

7. Secondary Antibody (day 2)
● After 2h, wash the plates 3x with PBS-T using the automated plate washer.
● Dilute anti-human IgG (Fab specific) HRP labeled secondary antibody 1:3000 in PBS-T containing 1% milk. Prepare at least 5 ml per plate.
● Add 50 μl to all wells of the plate using a multichannel pipette. Be sure to avoid touching the tips of the pipette to the walls of the well to avoid carry over and high background signals.
● Start the timer for 1h (stay in a range of 50min to 65min) as soon as the secondary antibody has been added to the first plate. Place plates in a 20°C (RT) incubator.

8. Plate development and reading (day 2)
● After 1h, wash plates 3x with PBS-T using an automated plate washer.
● Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1 gold + 1 silver tablet) dissolved in 20 ml WFI can be used for 2 plates.
● Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until ready to start adding to the plates (needs to be prepared fresh right before use).
● Add 100 μl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has been added to the first row on the first plate. Do not exceed 10 minutes of developing before stopping the reaction.
● To stop the reaction after exactly 10 minutes, add 50 μl of 3M HCl to all wells.
● Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after adding HCl) and record data.
● Samples that exceed certain OD_{490} cutoff value (proposed cutoff: OD_{490} = 0.15-0.2 or mean of negative controls plus 3 times the standard deviation of the negative controls) are assigned presumptive positive and will be tested in confirmatory ELISA using full-length spike protein.
● OD_{490} cutoff has to be experimentally determined and depends on assay background 404 and noise.

B - Spike confirmatory ELISA

1. Coating ELISA plates (day 1)
● Thaw the required number of vials of antigen (SARS-CoV-2 **Spike** protein) to coat 96-well microtiter ELISA plates at a concentration of 2 µg/ml. Once thawed, mix by gently vortexing vial before diluting in 1X PBS.
● Prepare approximately 5 mL for each plate to be coated.
● Coat plates with 50 µl of diluted protein per well using a multichannel pipette and a reservoir. Lightly tap plates against surface to ensure protein is evenly coating the bottom of every well.
● Incubate at 4°C overnight. Plates can likely be stored in 4°C for up to 1 week but this needs to be validate locally to ascertain that it does not change rhe results.

2. Block ELISA plate (day 2)
● Calculate to prepare at least 30 ml of blocking solution per plate.
● Blocking solution consists of PBS-T + 3% milk powder (weight/volume).
● Using an automated plate washer, wash coated ELISA plates 3x with PBS-T.
● Add 200 µl blocking solution to all wells of the plates, starting the timer for 1 h (do not exceed 4h) after completing the first plate. Place plates in a 20°C (RT) incubator.

3. Pre-diluting samples (day 2)
● Retrieve 1:5 pre-diluted samples from Part A to be tested and confirmed (samples that are above certain threshold in RBD screening ELISA based on a set OD_{490} value –see end of **A**).

4. Serial dilution (day 2)
● Calculate and prepare at least 20 ml of PBS-T + 1% mi k powder (weight/volume) per plate.
● After blocking incubation, remove plates from the room temperature incubator and throw off the blocking solution. Tap the plates dry on a kimwipe.
● Using a multichannel pipette, add 120 µl of PBS-T containing 1% milk to all wells of the plate.
● Leaving Columns 1 and 12 as BLANKS, add an extra 51 µl only to Columns 2 and 7 (=sample wells).
● Add 9 µl of 1:5 pre-diluted sample (final dilution 1:100 on the plate) to the first well in Column 2 and continue to add samples to all 8 wells. In Column 7, add samples to wells 1 through 6. Transfer positive and negative control into wells 7 and 8, respectively. See reference plate layout below.
● With the multichannel pipette, pipette up and down 4-6 times in Column 2 to mix. Discard these tips. With new tips, transfer 60 µl (3-fold dilution) from Column 2 to Column 3 and pipette up and down once 4-6 times to mix. Repeat this until Column 6; 450 discard 60 µl before Column 7.
● Taking fresh tips mix Column 7 by pipetting. Repeat the same process of transferring, mixing, and discarding tips from Columns 7-11. Once Column 11 is reached, discard 60 µl.
● Start timer for 2h (do not exceed 4h) once the first ELISA plate has been serially diluted.
● Place plates in a 20°C (RT) incubator.

5. Secondary Antibody (day 2)
● After 2h, wash the plates 3x with PBS-T using the automated plate washer.
● Dilute anti-human IgG (Fab specific) HRP labeled secondary antibody 1:3000 in PBS-T containing 1% milk. Prepare at least 5 ml per plate.
● Add 50 µl to all wells of the plate using a multichannel pipette. Be sure to avoid 466 touching the tips of the pipette to the walls of the well.
● Start the timer for 1 h (50 – 65 min) as soon as the secondary antibody has been 468 added to the first plate. Place plates in a 20°C (RT) incubator.

6. Plate development and reading (day 2)
● After 1h, wash plates 3x with PBS-T using an automated plate washer.
● Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1 gold + 1 silver tablet) dissolved in 20 mL WFI can be used for 2 plates.
● Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until 475 ready to start adding to the plates.
● Add 100 µl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has been added to the first row of the first plate. Do not exceed 10 minutes of developing before stopping the reaction.
● To stop the reaction after exactly 10 minutes, add 50 µl of 3M HCl to all wells.
● Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after adding HCl) and record data.
● True positive samples will have a signal higher than the negative control plus 3 standard deviations of the negative controls in at least two consecutive dilutions.

5.0 Statistics and Justification of Sample Size

Sample size justification

Commented [MOU8]: incomplete

For epidemiological studies it is important to capture a sample that is representative of the population at risk to understand the extent of the virus spread and to reduce the margin or

error. Due to the fact that this is a virus newly introduced to humanity, initial seroprevalence in the population is assumed to be minimal thus the entire population of patients and workforce is considered at risk.

Data analysis

Several epidemiological parameters will be evaluated including age-specific cumulative incidence which is defined as the proportion of individuals per age strata that are seropositive for COVID19 infection taking into account any difference the age stratification of the participants and the overall population. In addition, the proportion of asymptomatic cases over total number of confirmed cases at MD Anderson will be determined. Specifically, in cancer patients, we will quantify the presence of antibodies in confirmed COVID19 cases and compare it to the general population, and for patients with repeated samples, the change in levels of SARS-CoV-2 antibodies will be determined. Lastly, the case fatality ratio defined as the proporteion of individuals with fatal outcome for COVID19 infection will be established. This may require extended follow-up to determine the outcome of infection among those tested.

6.0 Procedure to Obtain Informed Consent

> **Commented [MOU9]:** Do we need IC if we are using residual samples and others collected under APOLLO?

7.0 Data Confidentiality

Data will be available to the PI and people directly involved with the collection and analysis of data related to this project. IRB approval will be obtained for any exchange of data within and outside of MD Anderson.

Collection of Identifiers:
Identifiers (name, dates, phone number, email address and MRN) will be collected. Identifiers (other than dates) will be replaced by study numbers in the analytic file. The key linking these numbers will be retained in a locked file or stored on an encrypted server behind the firewall, by the investigator's designated personnel. Dates will be retained as a limited data set. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval.

Training of Personnel:
All MD Anderson personnel will be fully trained to maintain the patient health information confidentially. Training will be documented as required by institutional policy.

Data Storage:
The PI and research staff will attempt to minimize risk through only storing information containing subject identifiers in locked file storage, and on password-protected computers,

on encrypted servers behind an institutional firewall and according to current institutional and federal data security requirements. In addition, access to patient identifiers will be limited to the minimum number of necessary research personnel, and only to those research personnel directly involved with obtaining patient information and assigning random study identifiers. Keys containing information linking study subjects to personal identifiers will be maintained in locked storage for paper records or behind institutionally approved firewall and electronic security measures for electronic keys, and available ONLY to the PI and research personnel directly involved in creating random study identifiers. Information containing subject personal identifiers will not be removed from MD Anderson Cancer Center without IRB approval and will not be shared in publications or reports concerning this research study.

Data Sharing:
Study data will not be shared with any individuals or entities that are not involved in the study. No identifying information will be shared with outside collaborating sites or outside collaborating research staff without prior IRB approval and a data use or material transfer agreement has been implemented. Sharing of data will be done after approval of the PI and only by secure mechanisms, as approved by MD Anderson Information Security.

Final Disposition of Study Records:
These data will be used for this research study. Data that is in hard-copy form will be retained on site until the study is terminated, and may be stored indefinitely, per institutional standards, in long-term off-site storage with an MD Anderson approved, secured contract site. Electronic data will be retained indefinitely on MD Anderson servers behind the institutional firewall. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval. Study data and paper records will not be destroyed but will be retained permanently.

8.0 References

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TITLE: Evaluating the seroprevalence of SARS-CoV02 antibodies in an asymptomatic or subclinical population at MD Anderson Cancer Center

Study Chair: Nadim Ajami
Co-Chairs: Eleonora Dondossola
Internal Collaborators: Padmanee Sharma, Jennifer Wargo, Alex Lazar
External Collaborators: Florian Krammer, Pedro Piedra
Department: Genomic Medicine
Phone:
Unit:

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PROTOCOL APPLICATION

Participant Population

Expected age range of participants:
18 and above

Will this include embryonic stem cells?
No

Does this research include MDACC employees as participants?
Yes

Total expected number of participants is 5,000 from 04/01/2020 through 04/01/2021. These are COVID19 patients or persons under investigation who undergo laboratory testing for COVID19 and where blood samples are drawn for standard of care procedures.

Specimen Type and Collection Type:
Residual blood serum samples and prospectively collected samples

Total # of Specimens, Volume, Frequency:

One sample per patient or as many as available up to 25,000 patient and employee's samples.

For residual blood serum samples, a minimum of 100 uL will be required. For prospectively collected samples, 5-10 mL of blood will be drawn per institutional standards.

List the source of specimens/data (Select all that apply):
Residual blood serum samples will be obtained from the Department of Pathology and Laboratory Medicine.
Prospectively collected samples will be drawn using an institutional Z-code under the APOLLO protocol and processed and banked through the institutional tissue bank (ITB).
Relevant metadata from patients will be collected from the electronic medical record (EMR).

Data Profile

Types of data points that will be collected include:
Names or initials, medical record number, age, sex, medications, medical comorbidities, smoking history, dates of surgery and other treatments at MD Anderson including intravenous or oral antibiotics administered and/or prescribed, dates of followup, results of imaging/pathology/labwork, date of recurrence/progression, and date of death.

Specimens/data sharing
Specimens/de-identified data will be shared with entities, persons, or organizations outside of MD Anderson only with proper approval and material transfer agreement (MTA).

Confidentiality
Will there be a link to identify subjects?
YES-PHI will be collected; however, study ID's will be used to de-identify data whenever possible. however only made available to the PI and mid-level providers/research/clinical fellow participating in the coordination of this research study.

Will identifiable data be made available to anyone other than the PI?
YES-Research/clinical fellow and mid-level providers participating in the coordination of this research study

Compensation
Will subjects receive compensation (e.g. water bottles, t shirts, gift cards), or reimbursements (e.g., gas cards for travel, parking vouchers, bus fare, or hotel reimbursement?)
No

Informed Consent Considerations

Please select the appropriate response(s) regarding the informed consent process for this study (you may select more than one)
Protocol staff will obtain prospective written consent

1.0 Objectives

The **primary aim** of this research study is to **determine the seroprevalence of antibodies against SARS-CoV-2, the causing agent of COVID19, among our patient population and workforce**. This study represents the initial step to determine the rate of seroposivitiy in our community. The data resulting from this study will be used to guide the development of larger ongoing study to determine the seroprevalence of SARS-CoV-2 antibodies in our population over the next 5 years.

Outcome Measures:
Primary:
- Determine the extent of SARS-CoV-2 infection in our community by means of seropositivity.

Secondary:
- Determine the cumulative incidence of infection in our patient population and workforce.
- Determine the isotype of antibodies raised against surface antigens of SARS-CoV-2

2.0 Rationale

Severe acute respiratory disease coronavirus 2 (SARS-CoV-2) has caused a pandemic known as **Co**rona**vi**rus **D**isease 20**19** (COVID-19). Patient's symptoms range from no detectable signs of infection, to fever, cough, acute respiratory distress syndrome and pneumonia that can lead to death in 1.4% of cases[1] across the general population and up to 8-20% in more vulnerable populations (i.e. >70 years of age, presence of coexisting disorders, including cancer)[2]. Several molecular assays are currently available to identify positive patients by directly detecting viral RNA in respiratory samples through real time reverse transcriptase-PCR. However, these assays have to be performed during a narrow window following infection otherwise the virus cannot longer be detected. Thus, testing of asymptomatic people has been challenging due to resource management and prioritization of symptomatic people, leaving many undiagnosed.

Irrespective of the absence of symptoms (which have been reported as high as 88%[3]), the infection leads to the generation of antibodies that can be detected in blood for months or longer following infection, offering an opportunity to determine the magnitude of cases via serologic testing. Although detecting antibodies against SARS-CoV-2 is indicative of prior exposure, it has diagnostic limitations for the acute stages of the disease as antibodies are generated 7-28 days after infection[4]. Based on recent data from Santa Clara County, California where seroprevalence was used determine the spread of the disease, results imply the infection is 50-85-fold more than the number of confirmed cases indicating that the infection is much more widespread than what direct virus testing suggests[5]. Therefore, a serologic test for COVID-19 would complement PCR-based results to calculate the proportion of infected individuals among our community of patients and our workforce.

Identifying those who have overcome the infection is needed to estimate illness prevalence in the overall population and have great implications in our understanding of how immunity develops, particularly in cancer patients.

Although limited information is available for SARS-CoV-2, protective immunity has been shown in primates after re-challenge to CoV2, with no evidence of viral loads and viral replication in all primary tissue compartments at five days post-reinfection[6]. In another study, seroconversion (IgG and IgM) in SARS-CoV-2 patients has been shown to occur by 7 days in 50% of individuals and 14 days in all of them[4]

Figure 1. Adapted from Wölfer et al.[4] Viral load kinetics, seroconverstion, and clinical observations in individual cases. Panels A to I correspond to 9 different cases studies. Dotted line, limit of quantification were performed in duplicate and the data presented are means of results. Experiment obtained by two laboratories independently.

(Fig. 2([4])). Similar results were confirmed by others ([7]) and evidence continues to grow. A more comprehensive study (n=175) detected neutralizing antibodies in >95% of patients positive to CoV-2 using a neutralization assay (Fig. 2)[8]

There are many formats in which serologic tests can be carried out but in this case an enzyme-linked immunosorbent assay (ELISA) is the most optimal option. ELISAs provide a relatively quick (>24 hours turnaround time) and inexpensive readout of antigen-antibody interaction that can be

Figure 2. Adapted from Wu et al.[7] Titers of neutralizing Abs against VSV, SARS-CoV, and SARS-CoV-2 pseudovirus in 26 COVID-19 recovered patient plasma. More than 95% developed antibodies above the detection limit (50 OD).

quantitative and specific for viral antigens and human antibodies. Previous work with SARS and MERS outbreaks, both caused by coronaviruses, identified an ELISA method that provided high sensitivity to detect the virus and was more efficient than other approaches.

No cross-reactivity was shown to other coronaviruses (OC43, HKU1, 229E, NL63) in ELISA assays recently developed to test SARS-CoV-2 (Fig. 3)[7,9,10]. With this novel coronavirus, initial seroprevalence in the population is assumed to be negligible due. Therefore, surveillance of antibody seropositivity in a population can allow inferences to be made about the extent of infection and about the cumulative incidence of infection in the population.

Fig. 4. Adapted from Okba et al.[6] 87%–100% of serum samples in control cohorts (blood donors, non-CoV infection, HCoV) were seropositive for endemic HCoVs (HCoV-HKU1, HCoV-OC43, HCoV-NL63, and HCoV-229E), as determined by the S1 protein microarray. No antibody cross-reactivity was shown with SARS-CoV-2.

Based on this evidence, epidemiologic studies are urgently needed to help uncover the burden of disease, in particular the rate of asymptomatic infections, and to get better estimates on illness and death. These studies can help identify the extent of virus spread in households, communities, and specific settings like our hospital and research buildings, which could help guide control measures. In the near future, serologic assays will be needed for evaluation of results of vaccine trials.

Hypothesis: we hypothesize that SARS-CoV-2 induces seroconversion in infected individuals stimulating the production of circulating antibodies against the virus.

3.0 Eligibility of Subjects

Adults of 18 years of age or older at MD Anderson Cancer Center with confirmed COVID19 laboratory diagnosis via direct virus testing (nasopharengeal samples, RT-qPCR) and persons under investigation (suspected COVID19 cases by symptomatology).

Inclusion: Adult subjects (≥18 years) with a laboratory confirmed diagnosis of COVID19 or under investigation for possible COVID19:

- Prospectively collected and residual blood serum samples from laboratory confirmed COVID19 cases or from presumed cases (persons under investigation) will be obtained from the Institutional Tissue Bank (ITB) at MD Anderson Cancer Center.

Exclusion: A subject will not be eligible for inclusion in this study if any of the following criteria apply:
- Age <18 years

4.0 Research Plan and Methods

The seroepidemiological investigation for COVID19 virus among MD Anderson is intended to provide key epidemiological and serologic characteristic of the disease. The model employed will follow a longitudinal investigation with serial sampling in which samples will be drawn from a specific population (i.e. MD Anderson Cancer Center patients and potentially the workforce) but not necessarily from the same individuals at specific time points.

Blood serum samples collected from individuals with confirmed laboratory results for COVID19 and others presumed to have the disease but not diagnosed (persons under investigation) will be included. These samples are collected as part of standard of care procedures and residual volumes are stored at the Institutional Tissue Bank (ITB). We will use those samples to evaluate the presence and the quantity of antibodies against SARS-CoV-2 to determine seroposivitiy rates.

The immunoassay that will be used to study the seroprevalence of antibodies against SARS-CoV-2 is being developed in collaboration with Florian Krammer, Professor of Vaccinology at the Department of Microbiology at the Icahn School of Medicine at Mount Sinai, the Principal Investigator of the Sinai-Emory Multi-Institutional Influenza Vaccine Innovation Center (SEM-CIVIC), and member of the NIH-funded Centers for Excellence in Influenza Research and Surveillance (CEIRS).

The IgG ELISA developed by Dr. Krammer received Emergency Use Approval by the FDA on April 16 (https://www.fda.gov/media/137032/download). He has shared reagents and a protocol for the development of the assay with the Chair and Co-Chair of this study and such reagents are being further shared with Dr. Pedro Piedra at Baylor College of Medicine who is leading the local implementation of Dr. Krammers' protocol. Dr. Piedra is a Professor in the Department of Molecular Virology and Microbiology and his research is focused on influenza virus, respiratory syncytial virus, adenovirus viruses, human metapneumovirus, and the recently described SARS-CoV-2.

The surface glycoprotein of the virus, termed spike (S) protein, mediates attachment of the virus to human cells via its receptor binding domain (RBD) and subsequent fusion of viral and cellular membranes[11]. Antibodies that bind to the spike protein, and especially to the

RBD domain, can neutralize coronaviruses. This assay uses different recombinant spike protein preparations as antigen for the ELISA. It has been reported that individuals not exposed to SARS-CoV-2 are completely naïve for the spike protein and show no reactivity in an ELISA[7,9,10] test. It is, therefore, possible to distinguish between exposed/immune and naïve people.

As described by Amanat et al9, a two-step ELISA protocol for high throughput screening of human serum samples for antibodies binding to the spike protein of SARS-CoV-2 will be implemented. In this a two-step ELISA. The first step includes relatively high throughput screening of samples in a single serum dilution against recombinant RBD produced in mammalian cells (which expresses very well and therefore leads to a higher protein yield). This is followed by a second step in which positive samples from the first step undergo a confirmatory ELISA against the full length spike protein (which is harder to purify, therefore there is usually less available). For the second step a dilution curve is performed. The throughput of this assay, if only one operator is available, allows to perform screening ELISAs (760 samples/10 plates per run) and confirmatory ELISAs in (140 samples/10 plates per run) in a single day. Negative controls include serum pools of serum taken before 2020. Positive controls are convalescent samples from COVID19 patients or monoclonal antibodies (mAbs) like CR3022912,13. If no human sera or mAbs are available, mouse mAbs, mouse sera against SARS-CoV-2, order animal sera against SARS-CoVo2 or anti-his tag antibodies (the recombinant proteins used as antigens are his-tagged) can be used. However, in this case a different secondary antibody for the species from which the primary antibody is derived is needed for the positive control. The positive control exceeds an OD490 of the negative control plus 3 standard deviations of the negative controls up to, at least, a 1:150 dilution. ELISAs can be run with both serum and plasma.

4.1 Subjects

Patients undergoing laboratory testing for COVID19 diagnosis at MD Anderson Cancer Center from 04/1/2020 to 04/01/2021 will have their blood drawn for standard of care procedures. Residual samples collected in serum separator tubes (SST, i.e. gold or red/gray) or serum tubes (i.e. red cap) will be stored at the Institutional Tissue Bank and used for this research.

MD Anderson staff including healthcare workers and research staff presenting no symptoms for COVID19 will be enrolled under the APOLLO protocol to determine seroprevalence of antibodies against SARS-CoV-2. Samples will be collected in a similar way as for patients undergoing standard of care testing as explained above.

4.2 Sample Collection

Residual samples obtained from the Institutional Tissue Bank from laboratory confirmed COVID19 cases and persons under investigation (PUIs) and prospectilvey collected samples from MD Anderson staff will be assayed to detect and quantify antibodies against

the viral receptor binding domain (RBD) of the spike protein of SARS-CoV-2. Both serum and plasma samples can be tested for the presence of antibodies against SARS-CoV-2.

Potential Harms/Adverse events:
No potential harms or adverse events are anticipated other than the very minimal risk associated with blood draws. The primary benefit of the study is indirect in that data collected will help improve our understanding of COVID19.

Safety Monitoring:
This study will be monitored by the MDACC Data and Safety Monitoring Board (DSMB).

ELISA testing should be carried out in a facility with at least biosafety level 2 (BSL-2) capacity. All personnel involved in the investigation need to be trained in infection prevention and control procedures (standard contact and droplet precautions, as determined by national or local guidelines). These procedures should include proper hand hygiene and the correct use of surgical masks, if necessary, not only to minimize their own risk of infection when in close contact with infectious material, but also to minimize the risk of spread among other participants in the investigation.

4.3 Testing

A two-step enzyme-linked immunosorbent assay (ELISAs) based on recombinant antigens derived from the spike protein of CoV-2, including its receptor binding domain (RBD) and full length spike (S) protein will be used. Such antigens were used for testing 59 negative human control sera preCOVID-19, including people with previous confirmed coronavirus exposure (e.g. NL63) and four samples from CoV-2 positive patients. ELISAs proved sensitive and specific and allowed for identification of positive patients using human plasma/serum as early as three days post-symptom onset (12.5 µl of plasma or serum needed/test; 2 wells, in duplicate, 6.25 µl/well). As an advantage, these assays do not require handling of infectious virus, are amenable to scaling and only few microliters of plasma/serum are needed. The assay will have sensitivity of >95% and specificity of >95% and pre-pandemic sera are used as controls to test cross reactivity to other coronavirus.
As mentioned, this assay got Emergency Use Approval by the FDA.

Detailed Protocol (Adapted from Stadlbauer et al, A detailed protocol for a serological assay to detect SARS-CoV-2 1 seroconversion in humans: antigen production and test setup).

A - RBD Screening ELISA

1. Coating ELISA plates (day 1)
● Thaw the required number of vials of antigen (SARS-CoV-2 **RBD** protein) to coat 96-well microtiter ELISA plates at a concentration of 2 µg/ml. Once thawed, mix by gently vortexing vial before diluting in 1X PBS.
● Prepare approximately 5 mL for each plate to be coated.

● Coat plates with 50 µl of diluted protein per well using a multichannel pipette and a reservoir. Lightly tap plates against surface to ensure protein is evenly coating the bottom of every well.
● Incubate at 4°C overnight. Plates can be stored at 4°C for up to 1 week.
● Always keep a cover plate on top of coated plates during all steps of the protocol!

2. Heat inactivation of samples (day 1, this is a safety precaution)
● Set the water bath to 56°C. Once temperature is reached, place the serum/plasma samples in and start the timer for 1h immediately.
● Remove samples when the timer goes off. Do not leave samples at 56°C for longer than 1h. Store at 4°C overnight or until use.

3. Block ELISA plate (day 2)
● Calculate to prepare at least 30 ml of blocking solution per plate.
● Blocking solution consists of PBS-T + 3% milk powder (weight/volume).
● Using an automated plate washer, wash coated ELISA plates 3x with PBS-T.
● Add 200 µl blocking solution to all wells of the plates, starting the timer for 1h (do not exceed 4h) after completing the first plate. Place plates in a 20°C (RT) incubator.
Note: This step (and wherever a plate washer is needed below) can also be performed by washing plates with a multichannel pipette by hand if no plate washer is available.

4. Pre-diluting samples (day 2)
● In a biological safety cabinet, set up sterile Eppendorf tubes to pre-dilute serum samples 1:5.
● Add 40 µl of sterile 1X PBS to all tubes.
● Gently vortex serum sample to mix and add 10 µl to the Eppendorf tube, vortexing once more. Do this for all remaining samples including the positive and negative controls. *Volume not needed in this part A will be stored and used for part B.*

5. Dilution plate set-up (day 2)
● Calculate and prepare at least 30 ml of PBS-T + 1% milk powder (weight/volume).
● Prepare 1 dilution plate (separate flat bottomed cell culture plate) per antigen coated plate prepared.
● Add 180 µl of PBS-T containing 1% milk to all wells of the dilution plate (including blank wells)
● Leaving Columns 1 and 12 as blanks, add 20 µl of sample (or control) into the designated well. This results in a final serum dilution of 1:50.
● Continue until all samples and controls have been added to designated wells. See reference plate layout below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	(+) Ctrl.	(-) Ctrl.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Blank
B	Blank	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	...	...	...			Blank
C	Blank											Blank
D	Blank											Blank
E	Blank											Blank
F	Blank											Blank
G	Blank											Blank
H	Blank		...	...	...	Sample 73	Sample 74	Sample 75	Sample 76	(+) Ctrl.	(-) Ctrl.	Blank

6. Transfer serum dilution (day 2)
● After blocking incubation, remove plates from the room temperature incubator and throw off the blocking solution. Tap the plates dry on a kimwipe.
● Using a multichannel pipette, pipette up and down 4-6 times in the first row of dilution plate to mix.
● Transfer 100 µl to the corresponding rows in the ELISA plate. Change tips and continue to transfer second row to the ELISA plate.
● Start the timer for 2h as soon as all the rows have been transferred to the first ELISA 374 plate. (Do not exceed 4h)
● Place plates in a 20°C (RT) incubator.

7. Secondary Antibody (day 2)
● After 2h, wash the plates 3x with PBS-T using the automated plate washer.
● Dilute anti-human IgG (Fab specific) HRP labeled secondary antibody 1:3000 in PBS-T containing 1% milk. Prepare at least 5 ml per plate.
● Add 50 µl to all wells of the plate using a multichannel pipette. Be sure to avoid touching the tips of the pipette to the walls of the well to avoid carry over and high background signals.
● Start the timer for 1h (stay in a range of 50min to 65min) as soon as the secondary antibody has been added to the first plate. Place plates in a 20°C (RT) incubator.

8. Plate development and reading (day 2)
● After 1h, wash plates 3x with PBS-T using an automated plate washer.
● Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1 gold + 1 silver tablet) dissolved in 20 ml WFI can be used for 2 plates.
● Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until ready to start adding to the plates (needs to be prepared fresh right before use).
● Add 100 µl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has been added to the first row on the first plate. Do not exceed 10 minutes of developing before stopping the reaction.
● To stop the reaction after exactly 10 minutes, add 50 µl of 3M HCl to all wells.

● Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after adding HCl) and record data.
● Samples that exceed certain OD_{490} cutoff value (proposed cutoff: OD_{490} = 0.15-0.2 or mean of negative controls plus 3 times the standard deviation of the negative controls) are assigned presumptive positive and will be tested in confirmatory ELISA using full-length spike protein.
● OD_{490} cutoff has to be experimentally determined and depends on assay background 404 and noise.

B - Spike confirmatory ELISA

1. Coating ELISA plates (day 1)
● Thaw the required number of vials of antigen (SARS-CoV-2 **Spike** protein) to coat 96-well microtiter ELISA plates at a concentration of 2 µg/ml. Once thawed, mix by gently vortexing vial before diluting in 1X PBS.
● Prepare approximately 5 mL for each plate to be coated.
● Coat plates with 50 µl of diluted protein per well using a multichannel pipette and a reservoir. Lightly tap plates against surface to ensure protein is evenly coating the bottom of every well.
● Incubate at 4°C overnight. Plates can likely be stored in 4°C for up to 1 week but this needs to be validate locally to ascertain that it does not change rhe results.

2. Block ELISA plate (day 2)
● Calculate to prepare at least 30 ml of blocking solution per plate.
● Blocking solution consists of PBS-T + 3% milk powder (weight/volume).
● Using an automated plate washer, wash coated ELISA plates 3x with PBS-T.
● Add 200 µl blocking solution to all wells of the plates, starting the timer for 1 h (do not exceed 4h) after completing the first plate. Place plates in a 20°C (RT) incubator.

3. Pre-diluting samples (day 2)
● Retrieve 1:5 pre-diluted samples from Part A to be tested and confirmed (samples that are above certain threshold in RBD screening ELISA based on a set OD_{490} value –see end of **A**).

4. Serial dilution (day 2)
● Calculate and prepare at least 20 ml of PBS-T + 1% milk powder (weight/volume) per plate.
● After blocking incubation, remove plates from the room temperature incubator and throw off the blocking solution. Tap the plates dry on a kimwipe.
● Using a multichannel pipette, add 120 µl of PBS-T containing 1% milk to all wells of the plate.
● Leaving Columns 1 and 12 as BLANKS, add an extra 51 µl only to Columns 2 and 7 (=sample wells).
● Add 9 µl of 1:5 pre-diluted sample (final dilution 1:100 on the plate) to the first well in Column 2 and continue to add samples to all 8 wells. In Column 7, add samples to wells 1 through 6. Transfer positive and negative control into wells 7 and 8, respectively. See reference plate layout below.
● With the multichannel pipette, pipette up and down 4-6 times in Column 2 to mix. Discard these tips. With new tips, transfer 60 µl (3-fold dilution) from Column 2 to Column 3 and pipette up and down once 4-6 times to mix. Repeat this until Column 6; 450 discard 60 µl before Column 7.
● Taking fresh tips mix Column 7 by pipetting. Repeat the same process of transferring, mixing, and discarding tips from Columns 7-11. Once Column 11 is reached, discard 60 µl.
● Start timer for 2h (do not exceed 4h) once the first ELISA plate has been serially diluted.

● Place plates in a 20°C (RT) incubator.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Sample 1					Sample 9					Blank
B	Blank	Sample 2					Sample 10					Blank
C	Blank	Sample 3					Sample 11					Blank
D	Blank	Sample 4					Sample 12					Blank
E	Blank	Sample 5					Sample 13					Blank
F	Blank	Sample 6					Sample 14					Blank
G	Blank	Sample 7					(+) Ctrl.					Blank
H	Blank	Sample 8					(-) Ctrl.					Blank

5. Secondary Antibody (day 2)
● After 2h, wash the plates 3x with PBS-T using the automated plate washer.
● Dilute anti-human IgG (Fab specific) HRP labeled secondary antibody 1:3000 in PBS-T containing 1% milk. Prepare at least 5 ml per plate.
● Add 50 µl to all wells of the plate using a multichannel pipette. Be sure to avoid 466 touching the tips of the pipette to the walls of the well.
● Start the timer for 1 h (50 – 65 min) as soon as the secondary antibody has been 468 added to the first plate. Place plates in a 20°C (RT) incubator.

6. Plate development and reading (day 2)
● After 1h, wash plates 3x with PBS-T using an automated plate washer.
● Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1 gold + 1 silver tablet) dissolved in 20 mL WFI can be used for 2 plates.
● Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until 475 ready to start adding to the plates.
● Add 100 µl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has been added to the first row of the first plate. Do not exceed 10 minutes of developing before stopping the reaction.
● To stop the reaction after exactly 10 minutes, add 50 µl of 3M HCl to all wells.
● Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after adding HCl) and record data.
● True positive samples will have a signal higher than the negative control plus 3 standard deviations of the negative controls in at least two consecutive dilutions.

5.0 Statistics and Justification of Sample Size

Sample size justification

For epidemiological studies it is important to capture a sample that is representative of the population at risk to understand the extent of the virus spread and to reduce the margin or error. Due to the fact that this is a virus newly introduced to humanity, initial seroprevalence in the population is assumed to be minimal thus the entire population of patients and workforce is considered at risk.

Data analysis

Several epidemiological parameters will be evaluated including age-specific cumulative incidence which is defined as the proportion of individuals per age strata that are seropositive for COVID19 infection taking into account any difference the age stratification of the participants and the overall population. In addition, the proportion of asymptomatic cases over total number of confirmed cases at MD Anderson will be determined. Specifically, in cancer patients, we will quantify the presence of antibodies in confirmed COVID19 cases and compare it to the general population, and for patients with repeated samples, the change in levels of SARS-CoV-2 antibodies will be determined. Lastly, the case fatality ratio defined as the proporteion of individuals with fatal outcome for COVID19 infection will be established. This may require extended follow-up to determine the outcome of infection among those tested.

6.0 Procedure to Obtain Informed Consent

No informed consent will be needed as these patients will be consented under the APOLLO protocol or residual samples will be used. Data will be obtained under the D3CODE protocol.

7.0 Data Confidentiality

Data will be available to the PI and people directly involved with the collection and analysis of data related to this project. IRB approval will be obtained for any exchange of data within and outside of MD Anderson.

Collection of Identifiers:
Identifiers (name, dates, phone number, email address and MRN) will be collected. Identifiers (other than dates) will be replaced by study numbers in the analytic file. The key linking these numbers will be retained in a locked file or stored on an encrypted server behind the firewall, by the investigator's designated personnel. Dates will be retained as a limited data set. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval.

Training of Personnel:
All MD Anderson personnel will be fully trained to maintain the patient health information confidentially. Training will be documented as required by institutional policy.

Data Storage:
The PI and research staff will attempt to minimize risk through only storing information containing subject identifiers in locked file storage, and on password-protected computers, on encrypted servers behind an institutional firewall and according to current institutional and federal data security requirements. In addition, access to patient identifiers will be limited to the minimum number of necessary research personnel, and only to those research personnel directly involved with obtaining patient information and assigning random study identifiers. Keys containing information linking study subjects to personal identifiers will be maintained in locked storage for paper records or behind institutionally approved firewall and electronic security measures for electronic keys, and available ONLY to the PI and research personnel directly involved in creating random study identifiers. Information containing subject personal identifiers will not be removed from MD Anderson Cancer Center without IRB approval and will not be shared in publications or reports concerning this research study.

Data Sharing:
Study data will not be shared with any individuals or entities that are not involved in the study. No identifying information will be shared with outside collaborating sites or outside collaborating research staff without prior IRB approval and a data use or material transfer agreement has been implemented. Sharing of data will be done after approval of the PI and only by secure mechanisms, as approved by MD Anderson Information Security.

Final Disposition of Study Records:
These data will be used for this research study. Data that is in hard-copy form will be retained on site until the study is terminated, and may be stored indefinitely, per institutional standards, in long-term off-site storage with an MD Anderson approved, secured contract site. Electronic data will be retained indefinitely on MD Anderson servers behind the institutional firewall. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval. Study data and paper records will not be destroyed but will be retained permanently.

8.0 References

1.   Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020.
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TITLE: Evaluating the seroprevalence of SARS-CoV02 antibodies in an asymptomatic or subclinical population at MD Anderson Cancer Center

Study Chair: Nadim Ajami
Co-Chairs: Eleonora Dondossola
Internal Collaborators: Roy Chemaly, Padmanee Sharma, Jennifer Wargo, Alex Lazar
External Collaborators: Florian Krammer, Pedro Piedra
Department: Genomic Medicine
Phone:
Unit:

Table of Contents

PROTOCOL APPLICATION

Participant Population

Expected age range of participants:
18 and above

Will this include embryonic stem cells?
No

Does this research include MDACC employees as participants?
Yes

Total expected number of participants is 5,000 from 04/01/2020 through 04/01/2021. These are COVID19 patients or persons under investigation who undergo laboratory testing for COVID19 and where blood samples are drawn for standard of care procedures.

Specimen Type and Collection Type:
Residual blood serum samples and prospectively collected samples

Total # of Specimens, Volume, Frequency:

One sample per patient or as many as available up to 5,000 patients' samples.

Commented [MOU1]: How does this sound?

For residual blood serum samples, a minimum of xx mL will be required. For prospectively collected samples, 5-10 mL of blood will be drawn per institutional standards.

Commented [MOU2]: Nadim – please work with Liz Burton on filling in these details. Check with Roy Chemaly as well

List the source of specimens/data (Select all that apply):
Residual blood serum samples will be obtained from the Department of Pathology and Laboratory Medicine.
Prospectively collected samples will be drawn using an institutional Z-code under the APOLLO protocol and processed and banked through the institutional tissue bank (ITB).
Relevant metadata from patients will be collected from the electronic medical record (Epic).

Data Profile

Types of data points that will be collected include:
Names or initials, medical record number, date of birth, gender, medications, medical comorbidities, smoking history, dates of surgery and other treatments at MD Anderson including intravenous or oral antibiotics administered and/or prescribed, dates of followup, results of imaging/pathology/labwork, date of recurrence/progression, and date of death.

Specimens/data sharing

Specimens/data will be shared with entities, persons, or organizations outside of MD Anderson only with proper approval and material transfer agreement (MTA)

Confidentiality

Will there be a link to identify subjects?
YES-PHI will be collected however only made available to the PI and mid-level providers/research/clinical fellow participating in this research study

Will identifiable data be made available to anyone other than the PI?
YES-Research/clinical fellow and mid-level providers participating in this research study

Compensation
Will subjects receive compensation (e.g. water bottles, t shirts, gift cards), or reimbursements (e.g., gas cards for travel, parking vouchers, bus fare, or hotel reimbursement?)
No

Informed Consent Considerations

Please select the appropriate response(s) regarding the informed consent process for this study (you may select more than one)
Protocol staff will obtain prospective written consent

1.0 Objectives

The **primary aim** of this research study is to **determine the seroprevalence of antibodies against SARS-CoV-2, the causing agent of COVID19, among our patient population and workforce**. This study represents the initial step to determine the rate of seroposivitiy in our community. The data resulting from this study will be used to guide the development of larger ongoing study to determine the seroprevalence of SARS-CoV-2 antibodies in our population over the next 5 years.

Outcome Measures:
Primary:
- Determine the extent of SARS-CoV-2 infection in our community by means of seropositivity.

Secondary:
- Determine the cumulative incidence of infection in our patient population and workforce.
- Determine the isotype of antibodies raised against surface antigens of SARS-CoV-2

2.0 Rationale

Severe acute respiratory disease coronavirus 2 (SARS-CoV-2) has caused a pandemic known as **Co**rona**vi**rus **D**isease 20**19** (COVID-19). Patient's symptoms range from no detectable signs of infection, to fever, cough, acute respiratory distress syndrome and pneumonia that can lead to death in 1.4% of cases[1] across the general population and up to 8-20% in more vulnerable populations (i.e. >70 years of age, presence of coexisting disorders, including cancer)[2]. Several molecular assays are currently available to identify positive patients by directly detecting viral RNA in respiratory samples through real time reverse transcriptase-PCR. However, these assays have to be performed during a narrow window following infection otherwise the virus cannot longer be detected. Thus, testing of asymptomatic people has been challenging due to resource management and prioritization of symptomatic people, leaving many undiagnosed.

Irrespective of the absence of symptoms (which have been reported as high as 88%[3]), the infection leads to the generation of antibodies that can be detected in blood for months or longer following infection, offering an opportunity to determine the magnitude of cases via serologic testing. Although detecting antibodies against SARS-CoV-2 is indicative of prior exposure, it has diagnostic limitations for the acute stages of the disease as antibodies are generated 7-28 days after infection[4]. Therefore, a serologic test for COVID-19 would complement PCR-based results to calculate the proportion of infected individuals among our community of patients and our workforce. Identifying those who have overcome the infection is needed to estimate illness prevalence in the overall population and have great implications in our understanding of how immunity develops, particularly in cancer patients.

Although limited information is available for SARS-CoV-2, protective immunity has been shown in primates after re-challenge to CoV2, with no evidence of viral loads and viral replication in all primary tissue compartments at five days post-reinfection[5]. In another study, seroconversion (IgG and IgM) in SARS-CoV-2 patients has been shown to occur by 7 days in 50% of individuals and 14 days in all of them[4]

Figure 1. Adapted from Wölfer et al.[4] Viral load kinetics, seroconverstion, and clinical observations in individual cases. Panels A to I correspond to 9 different cases studies. Dotted line, limit of quantification were performed in duplicate and the data presented are means of results. Experiment obtained by two laboratories independently.

(Fig. 2([4])). Similar results were confirmed by others ([6]) and evidence continues to grow. A more comprehensive study (n=175) detected neutralizing antibodies in >95% of patients positive to CoV-2 using a neutralization assay (Fig. 2)[7]

There are many formats in which serologic tests can be carried out but in this case an enzyme-linked immunosorbent assay (ELISA) is the most optimal option. ELISAs provide a relatively quick (>24 hours turnaround time) and inexpensive readout of antigen-antibody interaction that can be

Figure 2. Adapted from Wu et al.[7] Titers of neutralizing Abs against VSV, SARS-CoV, and SARS-CoV-2 pseudovirus in 26 COVID-19 recovered patient plasma. More than 95% developed antbodies above the detection limit (50 OD).

quantitative and specific for viral antigens and human antibodies. Previous work with SARS and MERS outbreaks, both caused by coronaviruses, identified an ELISA method that provided high sensitivity to detect the virus and was more efficient than other approaches. No cross-reactivity was shown to other coronaviruses (OC43, HKU1, 229E, NL63) in ELISA assays recently developed to test SARS-CoV-2 (Fig. 3)[6,8,9]. With this novel coronavirus, initial seroprevalence in the population is assumed to be negligible due. Therefore,

surveillance of antibody seropositivity in a population can allow inferences to be made about the extent of infection and about the cumulative incidence of infection in the population.

Fig. 4. Adapted from Okba et al. [6] 87%–100% of serum samples in control cohorts (blood donors, non-CoV infection, HCoV) were seropositive for endemic HCoVs (HCoV-HKU1, HCoV-OC43, HCoV-NL63, and HCoV-229E), as determined by the S1 protein microarray. No antibody cross-reactivity was shown with SARS-CoV-2.

Based on this evidence, epidemiologic studies are urgently needed to help uncover the burden of disease, in particular the rate of asymptomatic infections, and to get better estimates on illness and death. These studies can help identify the extent of virus spread in households, communities, and specific settings like our hospital and research buildings, which could help guide control measures. In the near future, serologic assays will be needed for evaluation of results of vaccine trials.

Hypothesis: we hypothesize that SARS-CoV-2 induces seroconversion in infected individuals stimulating the production of circulating antibodies against the virus.

3.0 Eligibility of Subjects

Inclusion: Adult subjects (≥18 years) with a laboratory confirmed diagnosis of COVID19 or under investigation for possible COVID19:
- Residual blood serum samples from laboratory confirmed COVID19 cases or from presumed cases (persons under investigation) will be obtained from the Institutional Tissue Bank (ITB) at MD Anderson Cancer Center

Exclusion: A subject will not be eligible for inclusion in this study if any of the following criteria apply:
- Samples kept in suboptimal conditions will be excluded from this study.

4.0 Research Plan and Methods

The seroepidemiological investigation for COVID19 virus among MD Anderson is intended to provide key epidemiological and serologic characteristic of the disease. The model employed will follow a repeated cross-sectional investigation in which samples will be drawn from a specific population (i.e. MD Anderson Cancer Center patients and potentially the workforce) but not necessarily from the same individuals each time. The timing of the study will depend on our ability to return to the research laboratories.

Blood serum samples collected from individuals with confirmed laboratory results for COVID19 and others presumed to have the disease but not diagnosed (persons under investigation) will be included. These samples are collected as part of standard of care procedures and residual volumes are stored at the Institutional Tissue Bank (ITB). We will use those samples to evaluate the presence and the quantity of antibodies against SARS-CoV-2 to determine seroposivitiy rates.

The immunoassay that will be used to study the seroprevalence of antibodies against SARS-CoV-2 is being developed in collaboration with Florian Krammer, Professor of Vaccinology at the Department of Microbiology at the Icahn School of Medicine at Mount Sinai, the Principal Investigator of the Sinai-Emory Multi-Institutional Influenza Vaccine Innovation Center (SEM-CIVIC), and member of the NIH-funded Centers for Excellence in Influenza Research and Surveillance (CEIRS).

The IgG ELISA developed by Dr. Krammer received Emergency Use Approval by the FDA on April 16 (https://www.fda.gov/media/137032/download). He has shared reagents and a protocol for the development of the assay with the Chair and Co-Chair of this study and such reagents are being further shared with Dr. Pedro Piedra at Baylor College of Medicine who is leading the local implementation of Dr. Krammers' protocol. Dr. Piedra is a Professor in the Department of Molecular Virology and Microbiology and his research is focused on influenza virus, respiratory syncytial virus, adenovirus viruses, human metapneumovirus, and the recently described SARS-CoV-2.

The surface glycoprotein of the virus, termed spike (S) protein, mediates attachment of the virus to human cells via its receptor binding domain (RBD) and subsequent fusion of viral and cellular membranes[10]. Antibodies that bind to the spike protein, and especially to the RBD domain, can neutralize coronaviruses. This assay uses different recombinant spike protein preparations as antigen for the ELISA. It has been reported that individuals not exposed to SARS-CoV-2 are completely naïve for the spike protein and show no reactivity in an ELISA[6,8,9] test. It is, therefore, possible to distinguish between exposed/immune and naïve people.

As described by Amanat et al[8], a two-step ELISA protocol for high throughput screening of human serum samples for antibodies binding to the spike protein of SARS-CoV-2 will be implemented. In this a two-step ELISA. The first step includes relatively high throughput screening of samples in a single serum dilution against recombinant RBD produced in mammalian cells (which expresses very well and therefore leads to a higher protein yield). This is followed by a second step in which positive samples from the first step undergo a confirmatory ELISA against the full length spike protein (which is harder to purify, therefore there is usually less available). For the second step a dilution curve is performed. The throughput of this assay, if only one operator is available, allows to perform screening ELISAs (760 samples/10 plates per run) and confirmatory ELISAs in (140 samples/10 plates per run) in a single day. Negative controls include serum pools of serum taken before 2020. Positive controls are convalescent samples from COVID19 patients or monoclonal antibodies (mAbs) like CR30229[11,12]. If no human sera or mAbs are available, mouse mAbs, mouse sera against SARS-CoV-2, order animal sera against SARS-CoVo2 or anti-his tag antibodies (the recombinant proteins used as antigens are his-tagged) can be used. However, in this case a different secondary antibody for the species from which the primary antibody is derived is needed for the positive control. The positive control exceeds an OD_{490} of the negative control plus 3 standard deviations of the negative controls up to, at least, a 1:150 dilution. ELISAs can be run with both serum and plasma.

4.1 Subjects

Patients undergoing laboratory testing for COVID19 diagnosis at MD Anderson Cancer Center from 04/1/2020 to 04/01/2021 will have their blood drawn for standard of care procedures. Residual samples collected in serum separator tubes (SST, i.e. gold or red/gray) or serum tubes (i.e. red cap) will be stored at the Institutional Tissue Bank and used for this research.

MD Anderson staff including healthcare workers and research staff presenting no symptoms for COVID19 will be enrolled under the APOLLO protocol to determine seroprevalence of antibodies against SARS-CoV-2. Samples will be collected in a similar way as for patients undergoing standard of care testing as explained above.

Commented [MOU4]: Need to verify that blood samples are collected in red or red/grey cap tubes

4.2 Sample Collection

Residual samples obtained from the Institutional Tissue Bank from laboratory confirmed COVID19 cases and persons under investigation (PUIs) and prospectilvey collected samples from MD Anderson staff will be assayed to detect and quantify antibodies against the viral receptor binding domain (RBD) of the spike protein of SARS-CoV-2. Both serum and plasma samples can be tested for the presence of antibodies against SARS-CoV-2.

Potential Harms/Adverse events:
No potential harms or adverse events are anticipated other than the very minimal risk associated with blood draws. The primary benefit of the study is indirect in that data collected will help improve our understanding of COVID19.

Safety Monitoring:
This study will be monitored by the MDACC Data and Safety Monitoring Board (DSMB).

ELISA testing should be carried out in a facility with at least biosafety level 2 (BSL-2) capacity. All personnel involved in the investigation need to be trained in infection prevention and control procedures (standard contact and droplet precautions, as determined by national or local guidelines). These procedures should include proper hand hygiene and the correct use of surgical masks, if necessary, not only to minimize their own risk of infection when in close contact with infectious material, but also to minimize the risk of spread among other participants in the investigation.

4.3 Testing

A two-step enzyme-linked immunosorbent assay (ELISAs) based on recombinant antigens derived from the spike protein of CoV-2, including its receptor binding domain (RBD) and full length spike (S) protein will be used. Such antigens were used for testing 59 negative human control sera preCOVID-19, including people with previous confirmed coronavirus exposure (e.g. NL63) and four samples from CoV-2 positive patients. ELISAs proved sensitive and specific and allowed for identification of positive patients using human plasma/serum as early as three days post-symptom onset (12.5 µl of plasma or serum needed/test; 2 wells, in duplicate, 6.25 µl/well). As an advantage, these assays do not require handling of infectious virus, are amenable to scaling and only few microliters of plasma/serum are needed. The assay will have sensitivity of >95% and specificity of >95% and pre-pandemic sera are used as controls to test cross reactivity to other coronavirus.
As mentioned, this assay got Emergency Use Approval by the FDA.

Detailed Protocol (Adapted from Stadlbauer et al, A detailed protocol for a serological assay to detect SARS-CoV-2 1 seroconversion in humans: antigen production and test setup).

A - RBD Screening ELISA

1. Coating ELISA plates (day 1)
● Thaw the required number of vials of antigen (SARS-CoV-2 **RBD** protein) to coat 96-well microtiter ELISA plates at a concentration of 2 µg/ml. Once thawed, mix by gently vortexing vial before diluting in 1X PBS.
● Prepare approximately 5 mL for each plate to be coated.
● Coat plates with 50 µl of diluted protein per well using a multichannel pipette and a reservoir. Lightly tap plates against surface to ensure protein is evenly coating the bottom of every well.

● Incubate at 4°C overnight. Plates can be stored at 4°C for up to 1 week.
● Always keep a cover plate on top of coated plates during all steps of the protocol!

2. Heat inactivation of samples (day 1, this is a safety precaution)
● Set the water bath to 56°C. Once temperature is reached, place the serum/plasma samples in and start the timer for 1h immediately.
● Remove samples when the timer goes off. Do not leave samples at 56°C for longer than 1h. Store at 4°C overnight or until use.

3. Block ELISA plate (day 2)
● Calculate to prepare at least 30 ml of blocking solution per plate.
● Blocking solution consists of PBS-T + 3% milk powder (weight/volume).
● Using an automated plate washer, wash coated ELISA plates 3x with PBS-T.
● Add 200 µl blocking solution to all wells of the plates, starting the timer for 1h (do not exceed 4h) after completing the first plate. Place plates in a 20°C (RT) incubator.
Note: This step (and wherever a plate washer is needed below) can also be performed by washing plates with a multichannel pipette by hand if no plate washer is available.

4. Pre-diluting samples (day 2)
● In a biological safety cabinet, set up sterile Eppendorf tubes to pre-dilute serum samples 1:5.
● Add 40 µl of sterile 1X PBS to all tubes.
● Gently vortex serum sample to mix and add 10 µl to the Eppendorf tube, vortexing once more. Do this for all remaining samples including the positive and negative controls. *Volume not needed in this part A will be stored and used for part B.*

5. Dilution plate set-up (day 2)
● Calculate and prepare at least 30 ml of PBS-T + 1% mi k powder (weight/volume).
● Prepare 1 dilution plate (separate flat bottomed cell culture plate) per antigen coated plate prepared.
● Add 180 µl of PBS-T containing 1% mi k to all wells of the dilution plate (including blank wells)
● Leaving Columns 1 and 12 as blanks, add 20 µl of sample (or control) into the designated well. This results in a final serum dilution of 1:50.
● Continue until all samples and controls have been added to designated wells. See reference plate layout below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	(+) Ctrl.	(-) Ctrl.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Blank
B	Blank	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	...	...	...			Blank
C	Blank											Blank
D	Blank											Blank
E	Blank											Blank
F	Blank											Blank
G	Blank											Blank
H	Blank		...	...	...	Sample 73	Sample 74	Sample 75	Sample 76	(+) Ctrl.	(-) Ctrl.	Blank

6. Transfer serum dilution (day 2)
● After blocking incubation, remove plates from the room temperature incubator and throw off the blocking solution. Tap the plates dry on a kimwipe.
● Using a multichannel pipette, pipette up and down 4-6 times in the first row of dilution plate to mix.
● Transfer 100 μl to the corresponding rows in the ELISA plate. Change tips and continue to transfer second row to the ELISA plate.
● Start the timer for 2h as soon as all the rows have been transferred to the first ELISA 374 plate. (Do not exceed 4h)
● Place plates in a 20°C (RT) incubator.

7. Secondary Antibody (day 2)
● After 2h, wash the plates 3x with PBS-T using the automated plate washer.
● Dilute anti-human IgG (Fab specific) HRP labeled secondary antibody 1:3000 in PBS-T containing 1% milk. Prepare at least 5 ml per plate.
● Add 50 μl to all wells of the plate using a multichannel pipette. Be sure to avoid touching the tips of the pipette to the walls of the well to avoid carry over and high background signals.
● Start the timer for 1h (stay in a range of 50min to 65min) as soon as the secondary antibody has been added to the first plate. Place plates in a 20°C (RT) incubator.

8. Plate development and reading (day 2)
● After 1h, wash plates 3x with PBS-T using an automated plate washer.
● Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1 gold + 1 silver tablet) dissolved in 20 ml WFI can be used for 2 plates.
● Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until ready to start adding to the plates (needs to be prepared fresh right before use).
● Add 100 μl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has been added to the first row on the first plate. Do not exceed 10 minutes of developing before stopping the reaction.
● To stop the reaction after exactly 10 minutes, add 50 μl of 3M HCl to all wells.
● Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after adding HCl) and record data.
● Samples that exceed certain OD_{490} cutoff value (proposed cutoff: OD_{490} = 0.15-0.2 or mean of negative controls plus 3 times the standard deviation of the negative controls) are assigned presumptive positive and will be tested in confirmatory ELISA using full-length spike protein.
● OD_{490} cutoff has to be experimentally determined and depends on assay background 404 and noise.

B - Spike confirmatory ELISA

1. Coating ELISA plates (day 1)
● Thaw the required number of vials of antigen (SARS-CoV-2 **Spike** protein) to coat 96-well microtiter ELISA plates at a concentration of 2 µg/ml. Once thawed, mix by gently vortexing vial before diluting in 1X PBS.
● Prepare approximately 5 mL for each plate to be coated.
● Coat plates with 50 µl of diluted protein per well using a multichannel pipette and a reservoir. Lightly tap plates against surface to ensure protein is evenly coating the bottom of every well.
● Incubate at 4°C overnight. Plates can likely be stored in 4°C for up to 1 week but this needs to be validate locally to ascertain that it does not change rhe results.

2. Block ELISA plate (day 2)
● Calculate to prepare at least 30 ml of blocking solution per plate.
● Blocking solution consists of PBS-T + 3% milk powder (weight/volume).
● Using an automated plate washer, wash coated ELISA plates 3x with PBS-T.
● Add 200 µl blocking solution to all wells of the plates, starting the timer for 1 h (do not exceed 4h) after completing the first plate. Place plates in a 20°C (RT) incubator.

3. Pre-diluting samples (day 2)
● Retrieve 1:5 pre-diluted samples from Part A to be tested and confirmed (samples that are above certain threshold in RBD screening ELISA based on a set OD_{490} value –see end of **A**).

4. Serial dilution (day 2)
● Calculate and prepare at least 20 ml of PBS-T + 1% mi k powder (weight/volume) per plate.
● After blocking incubation, remove plates from the room temperature incubator and throw off the blocking solution. Tap the plates dry on a kimwipe.
● Using a multichannel pipette, add 120 µl of PBS-T containing 1% milk to all wells of the plate.
● Leaving Columns 1 and 12 as BLANKS, add an extra 51 µl only to Columns 2 and 7 (=sample wells).
● Add 9 µl of 1:5 pre-diluted sample (final dilution 1:100 on the plate) to the first well in Column 2 and continue to add samples to all 8 wells. In Column 7, add samples to wells 1 through 6. Transfer positive and negative control into wells 7 and 8, respectively. See reference plate layout below.
● With the multichannel pipette, pipette up and down 4-6 times in Column 2 to mix. Discard these tips. With new tips, transfer 60 µl (3-fold dilution) from Column 2 to Column 3 and pipette up and down once 4-6 times to mix. Repeat this until Column 6; 450 discard 60 µl before Column 7.
● Taking fresh tips mix Column 7 by pipetting. Repeat the same process of transferring, mixing, and discarding tips from Columns 7-11. Once Column 11 is reached, discard 60 µl.
● Start timer for 2h (do not exceed 4h) once the first ELISA plate has been serially diluted.
● Place plates in a 20°C (RT) incubator.

5. Secondary Antibody (day 2)
● After 2h, wash the plates 3x with PBS-T using the automated plate washer.
● Dilute anti-human IgG (Fab specific) HRP labeled secondary antibody 1:3000 in PBS-T containing 1% milk. Prepare at least 5 ml per plate.
● Add 50 μl to all wells of the plate using a multichannel pipette. Be sure to avoid 466 touching the tips of the pipette to the walls of the well.
● Start the timer for 1 h (50 – 65 min) as soon as the secondary antibody has been 468 added to the first plate. Place plates in a 20°C (RT) incubator.

6. Plate development and reading (day 2)
● After 1h, wash plates 3x with PBS-T using an automated plate washer.
● Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1 gold + 1 silver tablet) dissolved in 20 mL WFI can be used for 2 plates.
● Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until 475 ready to start adding to the plates.
● Add 100 μl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has been added to the first row of the first plate. Do not exceed 10 minutes of developing before stopping the reaction.
● To stop the reaction after exactly 10 minutes, add 50 μl of 3M HCl to all wells.
● Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after adding HCl) and record data.
● True positive samples will have a signal higher than the negative control plus 3 standard deviations of the negative controls in at least two consecutive dilutions.

5.0 Statistics and Justification of Sample Size

Sample size justification   Commented [MOU5]: incomplete

For epidemiological studies it is important to capture a sample that is representative of the population at risk to understand the extent of the virus spread and to reduce the margin or

error. Due to the fact that this is a virus newly introduced to humanity, initial seroprevalence in the population is assumed to be minimal thus the entire population of patients and workforce is considered at risk.

Data analysis

Several epidemiological parameters will be evaluated including age-specific cumulative incidence which is defined as the proportion of individuals per age strata that are seropositive for COVID19 infection taking into account any difference the age stratification of the participants and the overall population. In addition, the proportion of asymptomatic cases over total number of confirmed cases at MD Anderson will be determined. Specifically, in cancer patients, we will quantify the presence of antibodies in confirmed COVID19 cases and compare it to the general population, and for patients with repeated samples, the change in levels of SARS-CoV-2 antibodies will be determined. Lastly, the case fatality ratio defined as the proporteion of individuals with fatal outcome for COVID19 infection will be established. This may require extended follow-up to determine the outcome of infection among those tested.

6.0 Procedure to Obtain Informed Consent

> **Commented [MOU6]:** Do we need IC if we are using residual samples and others collected under APOLLO?

7.0 Data Confidentiality

Data will be available to the PI and people directly involved with the collection and analysis of data related to this project. IRB approval will be obtained for any exchange of data within and outside of MD Anderson.

Collection of Identifiers:
Identifiers (name, dates, phone number, email address and MRN) will be collected. Identifiers (other than dates) will be replaced by study numbers in the analytic file. The key linking these numbers will be retained in a locked file or stored on an encrypted server behind the firewall, by the investigator's designated personnel. Dates will be retained as a limited data set. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval.

Training of Personnel:
All MD Anderson personnel will be fully trained to maintain the patient health information confidentially. Training will be documented as required by institutional policy.

Data Storage:
The PI and research staff will attempt to minimize risk through only storing information containing subject identifiers in locked file storage, and on password-protected computers,

on encrypted servers behind an institutional firewall and according to current institutional and federal data security requirements. In addition, access to patient identifiers will be limited to the minimum number of necessary research personnel, and only to those research personnel directly involved with obtaining patient information and assigning random study identifiers. Keys containing information linking study subjects to personal identifiers will be maintained in locked storage for paper records or behind institutionally approved firewall and electronic security measures for electronic keys, and available ONLY to the PI and research personnel directly involved in creating random study identifiers. Information containing subject personal identifiers will not be removed from MD Anderson Cancer Center without IRB approval and will not be shared in publications or reports concerning this research study.

Data Sharing:
Study data will not be shared with any individuals or entities that are not involved in the study. No identifying information will be shared with outside collaborating sites or outside collaborating research staff without prior IRB approval and a data use or material transfer agreement has been implemented. Sharing of data will be done after approval of the PI and only by secure mechanisms, as approved by MD Anderson Information Security.

Final Disposition of Study Records:
These data will be used for this research study. Data that is in hard-copy form will be retained on site until the study is terminated, and may be stored indefinitely, per institutional standards, in long-term off-site storage with an MD Anderson approved, secured contract site. Electronic data will be retained indefinitely on MD Anderson servers behind the institutional firewall. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval. Study data and paper records will not be destroyed but will be retained permanently.

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The COVID-19 Pandemic – The Known and the Unknown
By Hagop Kantarjian, MD, Steven Kornblau, MD, Mary Alma Welch, MMSc, & Andrew DiNardo, MD

The COVID-19 (Corona Virus Disease-2019) world pandemic continues to spread its devastation, causing the death of thousands, decimating world economies, and destroying familiar societal fabrics. It is premature to estimate its ultimate toll in numbers of infections and deaths, but we know that the U.S. and most of the world are still in the early-mid phases of the pandemic, during which the prevalence (total number of infected people) increases by 33% daily, which means it doubles every 3 days (actually 2.3 x increase).[1]

The Spread of COVID-19

The first cases of COVID-19 were reported in China in mid-November 2019. The disease abated there in early March, after severe social restrictions and wide COVID-19 screening, isolation, and prevention. As of March 31, 2020, of about 1.3 billion citizens, only about 82,000 were infected (infection rate less than 0.01%), and 3,300 died (mortality rate 4%).[2] Some experts criticized China for its early delays and lack of transparency, and question the figures, particularly the number of deaths, which is thought to be 3 times higher.

Some countries had experience with previous regional epidemics like Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). They took the threat seriously, developing early COVID testing and wide screening, and implementing strict measures of contact tracing and isolation, and of social distancing. They were able to contain the COVID-19 spread earlier and more effectively: South Korea, Australia, Hong Kong, Japan, Singapore, Taiwan, and others. For example, South Korea has a population of 51 million. As of March 31, 2020, it has reported 9,800 infections (infection rate=0.02%) and 162 deaths (mortality rate1.6%). Australia, with a population of 25.5 million, has reported 4,600 infections (infection rate 0.01%) and 17 deaths (mortality rate 0.4%).

Countries that ignored the COVID-19 threat early on did poorly. Spain has a population of 47 million. As of March 31, 2020, it has reported 95,000 infections (infection rate 0.2%) and 8,200 deaths (a high mortality rate of 8.6%). Italy, with a population 60.5 million, has so far reported infections 102,000 (infection rate 0.17%) and 11,600 deaths (the highest mortality rate so far; 11.3%). Studies have shown that, in such outbreaks, a delay of control measures of 1 week may triple the epidemic size and prolong it by 1 month.[3]

The United States ignored the threat of COVID-19 early on. Because of different convictions, and fear of the possible economic impact of a viral pandemic, opinions concerning the potential severity and impact of COVID-19 were, until recently, divided. There is now consensus that the COVID-19 pandemic is a threat to the United States and the world. The United States had also significant delays (about 6-8 weeks) in early and wide COVID-19 testing, due to issues with developing the test,[4] and with implementing social distancing and restrictions. As of March 31, 2020, of 330 million Americans, 182,000 are now infected (infection rate 0.05%), and 3,600 have died (mortality rate 2%). The situation is evolving rapidly, and by the time this editorial is published (April 15, 2020 = 3 days x 5 doublings = 16x estimated increase in prevalence = 2.91 million infected cases in the United States), we can compare the estimated figure to the actual

numbers, in order to assess whether the measures taken (wider COVID-19 testing; social distancing) are working (flattening of the curve) or not.

Previous Viral Infections as Guidance

We need to dispel misconceptions, propagated perhaps by incorrect data interpretation, and by ideological or economic convictions. COVID-19 is not "like influenza." The influenza season of 2017-2018 infected 45 million Americans, and caused the death of 61,000, a mortality rate of about 0.13%.[5] The estimated mortality rate from COVID-19 is so far 4% to 5%, a mortality rate 30-40 times higher than that of influenza. Of interest is the difference in mortality rates in different geographies. The mortality rate is so far 5% worldwide, 2% or less in some countries, and more than 5% in others. This may relate to different factors: early and wide testing; social distancing; weather conditions; medical capacities; effect of previous lung damage, from smoking for example; genetic variations; reporting; and others. For example, wide COVID-19 testing increases the denominator of people tested to include subclinical and milder cases; this results in a lower reported mortality rate. Limited screening (for example, by the CDC criteria) would include more symptomatic and severe cases; this results in a higher reported mortality rate. The mortality rate is of particular concern to oncologists and their patients with cancer, in whom, due to their immunocompromised state, the mortality rate could be as high as 30% to 40%, based on studies of small numbers of patients from China.[6]

What can we learn from previous viral epidemics, in particular the most devastating one, the 1918-1920 "Spanish influenza" pandemic? This viral pandemic recurred seasonally in 3 waves over 2 years, infected a third of the world population (500 million of 1.5 billion), and caused the death of an estimated 50 million worldwide (mortality rate 10% over the 3 waves).[7] (Unlike other epidemics where the virus generally mutates to a milder form, most of the deaths occurred during the second wave (thought to be due to a more virulent form of the original virus). Unlike today, however, there were no vaccines to mitigate the spread of the virus, no safe and effective anti-viral drugs, no antibiotics to treat secondary infections that increase mortality, no scientific progress as exists today, and no sophisticated medical facilities (with specialized medical personnel, ventilators, dialysis machines). What is known is that states and municipalities that implemented preventive measures had lower mortality rates than ones that did not.[7]

The figures from the Spanish influenza pandemic are in stark contrast with the second example: the swine flu of 1976.[8] It was estimated then that a flu epidemic was occurring every 11 years. Since one had not happened in 39 years, and because cases of swine flu were reported at an army base—Fort Dix, NJ—a massive vaccination campaign of 45 million Americans followed. The flu epidemic never happened, a handful of reported deaths were attributed to the newly identified Legionnaire's disease, and 50-450 cases of neurologic problems (Guillain-Barre syndrome) were attributed to the vaccine.[8] This example has been highlighted to advocate for a conservative approach (of course, not applicable to COVID-19).

The third example, the swine flu of 2009, falls in between the 2 previous extreme examples. The virus infected 60 million Americans and caused the death of 12,469, a mortality rate of 0.02%. A vaccine was available then, and because political ideology influenced vaccination rates, there was a higher mortality in some states, which also had lower vaccination rates.[9] This example highlights the importance of a readily available, safe and effective vaccine to mitigate the spread of a viral epidemic and to reduce mortality.

Updated Information on COVID-19

Can we predict how the COVID-19 pandemic will behave and its ultimate death toll in the United States and worldwide? We know COVID-19 is a highly contagious airborne virus that has the ability to survive on inert surfaces from 2-3 hours to as long as 8 days depending on the ambient temperature and other "virus unfavorable "conditions.[10] Seasonal influenza transmits from 1 person to 1.2; COVID-19 transmits from 1 person to 6-7 without precautions, and to 2-3 with precautions. A person infected with COVID-19 may remain asymptomatic for a median of 3 to 7 days (occasionally for up to 2 weeks). Many infected individuals may have minimal or no symptoms. Infectivity is highest around 5-7 days from exposure (at the time of "subclinical disease," within 48 hours of developing clinical symptoms.[11] From the Chinese experience, 80% 0f COVID-19 spread is caused by the 10%-20% of infected people who have minimal or no symptoms, because of lack of wide COVID-19 testing.[12] This why COVID-19 is so contagious and difficult to control if the current CDC criteria (cough and shortness of breath, fever, and known exposure) are used for testing. The clinical disease and infectivity last 1-2 weeks, but there are outliers with clinical findings (and infectivity) lasting for up to 3-4 weeks (5% or less). Hence the need for prolonged city lockdowns. In dealing with historical epidemics (plague, smallpox, cholera, typhoid), our ancestors implemented isolation procedures called "quarantaine" (40 days in French; presumably starting with the bubonic plague of the 14th century). These quarantines worked empirically in abating the spread of historical epidemics. We hope COVID-19 will not require 6-week lockdowns. The quarantine durations may be guided by the flattening prevalence curves from their initial exponential growth.

 It is now suspected that COVID-19 may recur in seasonal waves, but it is unknown if a second wave will be more virulent (like with the Spanish influenza) or less virulent (like other viruses). Several studies reported that COVID-19 spread may be slowed by warmer weather.[13] Based on coronavirus studies in Macaque monkeys, COVID-19 infections may produce immunity lasting for up to 1 year. Thus, it is possible that even if it recurs, herd immunity may prevent or abate it. Or, we may have by then safe and effective vaccines and anti-COVID-19 antiviral drugs. So the long-term health care impact of the COVID-19 pandemic may be less severe than that of the 1918-1920 Spanish influenza. However, the long-term economic impact may be worse because of the globalization and interconnection of world economies. The economic impact of the 1918-20 Spanish influenza was closely intertwined with the post-World War I consequences, so it cannot be assessed as a stand-alone event. Today, the world is interconnected, economies are global and depend on many now giant industries that did not exist in 1920: oil, global tourism, air, sea and land transportations, sports, technology, health care and entertainment. Some economic experts are comparing the current situation to the 1929 economic meltdown. But some industries that address the problems created by COVID-19 (virtual connections and meetings, software technologies, health care) may do better than others (tourism, transportations, physical entertainment events, arts, and sports)

Addressing COVID-19 in the United States

The impact of COVID-19 in the United States is evolving daily, and different regions are affected in different ways. Some urban and metropolitan areas became early COVID-19 epicenters because of their geographies and characters: cultural norms and traditions related to the amount of social contact; human densities; extent of reliance on mass transit; weathers (warm and humid better); tourism; mass gatherings. Cities such as New York, Seattle, San Francisco,

and Chicago are in the midst of COVID-19 surges that are overwhelming their medical infrastructures: hospital beds, intensive care unit beds, ventilators, health care workers. Activation of the Defense Production Act will help secure needed supplies. New Orleans and Louisiana became the most recent and fastest growing COVID-19 epicenter, because, in addition to factors shared by other cities (human density, reliance on tourism, warm cultural connections), they just concluded the Mardi Gras festivities that attracted 1.4 million tourists over one week.

Delays in COVID-19 Testing

One of the important issues to address for the future is the delays and extent of COVID-19 testing in the United States. As stated earlier, a week delay in addressing epidemics issues can increase and prolong significantly their effects. The delay in COVID-19 testing might end up being the "Achilles heel" of the United States' response to the pandemic.

Several analyses detailed the reasons behind the COVID-19 testing delays, in order to understand how to avoid them in the future.[4, 14-18] There appear to be heroes in these narratives, for example Dr. Helen Y. Chu, an infectious disease expert from Seattle who faced bureaucratic hurdles when she tried to facilitate early testing. Delays were also attributed to regulatory hurdles and existing safety rules mandated by the FDA, CDC, and other federal government entities. These safety rules are needed for general medical issues we have faced in the past, but they may not be nimble enough to address a public health pandemic of disastrous proportions, one not encountered since 1918. As an example, the CDC decided, as is it had done historically, to develop its own COVID-19 test. The initial tests were not reproducible, resulting in critical delays. A second example is the CDC testing criteria that required that individuals have fever, cough and shortness of breath, and document exposure to COVID-19 infected persons. These criteria may too strict and not effective in preventing disease spread.

Modeling Expectations of COVID-19

The CDC proposed different predictive models for COVID-19 outcomes. These assumed wide ranges of infection rates (20% to 60%) and of mortality rates (0.5% to 4%), and produced vastly different numbers. Based on the Diamond Princess Boat cruise data (3,300 crew members and passengers, 712 infected, 10 deaths), the infection rate may be about 20% and mortality rate 1.5%. This model is geographically dense and restricted, and may not represent the experience in less dense groups and with wide COVID-19 testing and social distancing and isolation. The current infection rates in different countries are so far less than 1%-5%, but the mortality rates range from less than 1% to more than 10%. If we extrapolate the Princess Boat data, the first COVID-19 wave may infect about 70 million Americans and cause the death of almost a million. But if we use the so far more common infection rates of 1% to 5% seen in many other countries, and a 1%-2% mortality rate, then the expectation would be that 3 million to 16 million Americans may be infected, and 60,000 to 320,000 may die. This assumes immediate and wide COVID testing and severe social restrictions. These figures need to be seriously considered as we, Americans, deliberate the value of our individual freedoms (ignoring proper hygiene, social distancing, avoiding large gatherings, etc.) versus societal obligations (caring for the most vulnerable; older Americans).

The figures cited above can be improved by multiple interventions. The most important "modifiers" are: 1) Earlier and broader testing of COVID-19; 2) strict preventive hygienic and societal measures (frequent hand washing; social distancing; avoiding large gatherings, crowds,

events, restaurants, theaters; staying at home if ill etc.); 3) safe and effective vaccines (human trials have started, and we hope vaccines may become be available before a potential second COVID-19 wave; and 4) effective antiviral therapies to treat COVID-19 (at least 50 drugs are now being tested). In essence, we hope for a relatively optimistic outlook for the COVID-19 pandemic in the United States, if all "modifiers" are realized as soon as possible.

Summary

What we must learn from COVID-19 is that anticipating the severity of the threat and preparing for it—developing early testing tools, early and broad COVID-19 testing, strict cautionary and preventive measures, and innovation (new vaccines and drugs)—are necessary to lessen the serious consequences of the pandemic on the world health care, societal fabrics, and economy. For now, we should prepare for the worst and hope for the best.

[[box text with photos]] **HAGOP KANTARJIAN, MD**, is a medical oncologist and a Special Fellow of Health Policies at the Baker Institute, Rice University. **STEVE KORNBLAU, MD**, is a medical oncologist in Houston. **MARY ALMA WELCH, MMSC**, is a physician assistant in Houston. **ANDREW DINARDO, MD**, is an infectious disease specialist in Houston. The opinions of the authors do not reflect those of the institutions with which they are affiliated.

The COVID-19 Pandemic

Call out

What we must learn from COVID-19 is that early and broad COVID-19 testing, strict cautionary and preventive measures, and innovation (new vaccines and drugs) are necessary in order to lessen the serious consequences of the COVID-19 pandemic on the world health care, societal fabrics, and economy.

Image
To come

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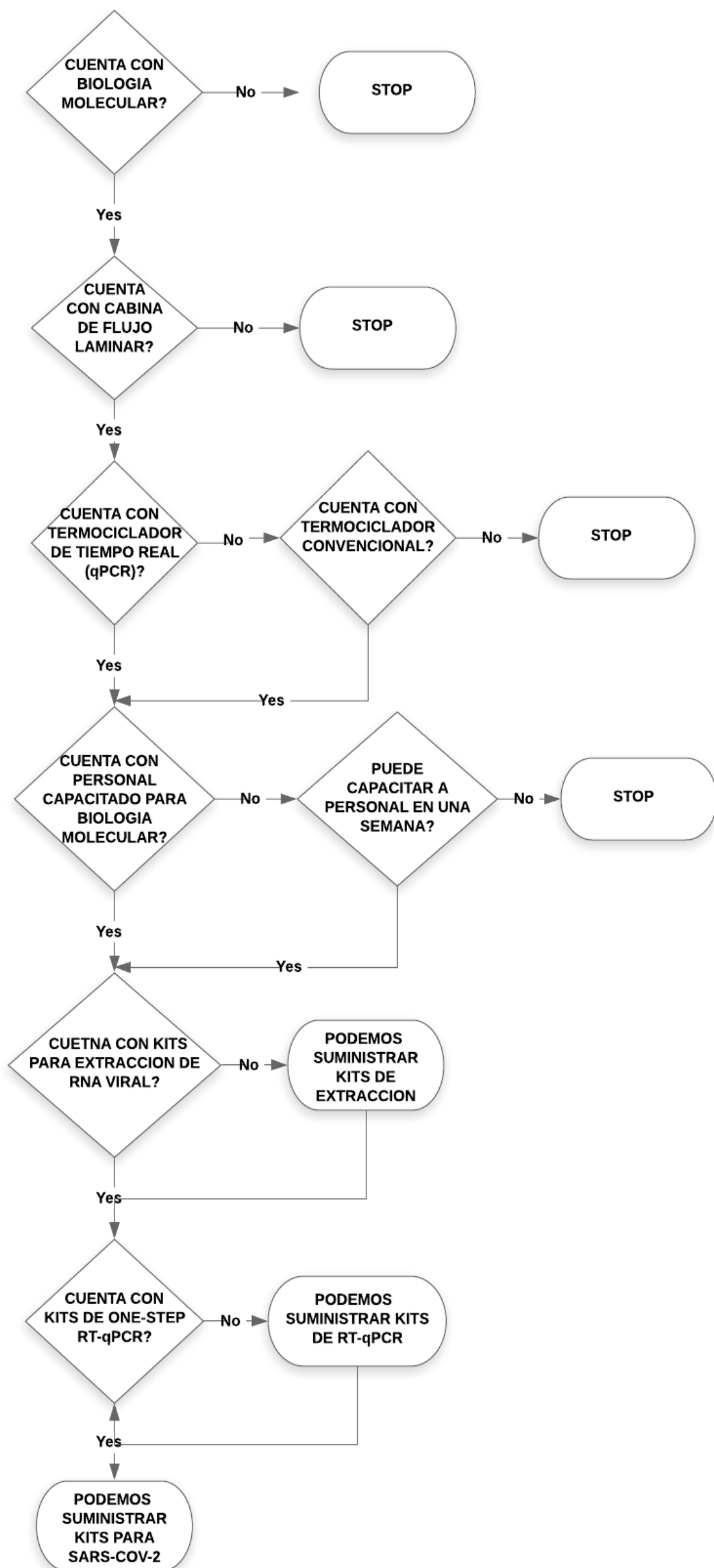
SPECIMENS

- Nasopharyngeal (preferred)
- Oropharyngeal
- Sputum

SPECIMEN COLLECTION AND MEDIA

Viral Transport Media (preferred)
- Copan: 305C, 307C, 360C and 519CS01*
- Puritan: UT-367, UT-317, UT-302*, UT-366** and UT-300***
- Hardy/Healthlink: 330CHL
- BD: 220526, 220258*, 220529, 220531
- DHI/Quidel: 330C.DHI and 503CS01.DHI
- Fisher Healthcare: 23001718, 23600952, 23600956, 23600950 and 23600957*
- PrimeStore MTM: LH-1-02 and LH-1-03***
  *flocked oropharyngeal swab
  **Polyester swab
  ***no swab

Liquid Aimes-based transport media

- Copan: 481C, 482C 480C* and 480CFA*
- Puritan: LA-117, LA-116-H and LA-100***
- BD: 220246, 220532 and 220245*
- ThermoFisher: R723481, R723482 and R723480*
- Hardy/Healthlink: 481C, 482C 480C* and 480CFA*
- VWR: 89136-656, 89136-658, 89136-654* and 76181-494*
- Fisher Healthcare: 23600901, 23600902, 23600900* and 23600905*

*flocked oropharyngeal swab
***no swab

Sterile saline solution

- ThermoFisher: R064430, R064432, R064434, R064436 and R064438
- Hardy/Healthlink: D185, K248, R45 and R55
- Edge Biologicals: T-0625 and T-0110f

VIRAL RNA EXTRACTION

- **Roche MagNA Pure LC**
  Kit: Roche MagNA Pure Total Nucleic Acid Kit
  Protocol: Total NA External_lysis
  Recommendation(s): Add 100 µL of sample to 300 µL of pre-aliquoted TNA isolation kit lysis buffer (total input sample volume is 400 µL). Elution volume is 100 µL.

- **Roche MagNA Pure Compact**
  Kit: Roche MagNA Pure Nucleic Acid Isolation Kit I
  Protocol: Total_NA_Plasma100_400
  Recommendation(s): Add 100 µL of sample to 300 µL of pre-aliquoted TNA isolation kit lysis buffer (total input sample volume is 400 µL). Elution volume is 100 µL.
- **Roche MagNA Pure 96**
  Kit: Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit
  Protocol: Viral NA Plasma Ext Lys SV Protocol
  Recommendation(s): Add 100 µL of sample to 350 µL of pre-aliquoted External Lysis Buffer (supplied separately) (total input sample volume is 450 µL). Proceed with the extraction on the MagNA Pure 96. (Note: Internal Control = None). Elution volume is 100 µL.
- **QIAGEN QIAcube**
  Kit: QIAGEN QIAamp® DSP Viral RNA Mini Kit or QIAamp® Viral RNA Mini Kit
  Recommendations: Utilize 140 µL of sample and elute with 100 µL of buffer.
- **QIAGEN**
  Kit: QIAGEN QIAamp® DSP Viral RNA Mini Kit or QIAamp® Viral RNA Mini Kit
  Recommendations: Utilize 100 µL of sample and elute with 100 µL of buffer or utilize 140 µL of sample and elute with 140 µL of buffer.
- **QIAGEN EZ1 Advanced XL**
  Kit: QIAGEN EZ1 DSP Virus Kit and Buffer AVL (supplied separately) for offboard lysis
  Card: EZ1 Advanced XL DSP Virus Card
  Recommendations: Add 120 µL of sample to 280 µL of pre-aliquoted Buffer AVL (total input sample volume is 400 µL). Proceed with the extraction on the EZ1 Advanced XL. Elution volume is 120 µL.
- **QIAGEN EZ1 Advanced XL**
  Kit: QIAGEN EZ1 Virus Mini Kit v2.0 and Buffer AVL (supplied separately) for offboard lysis
  Card: EZ1 Advanced XL Virus Card v2.0
  Recommendations: Add 120 µL of sample to 280 µL of pre-aliquoted Buffer AVL (total input sample volume is 400 µL). Proceed with the extraction on the EZ1 Advanced XL. Elution volume is 120 µL.
- **bioMérieux NucliSENS easyMAG Instrument**
  Protocol: General protocol (not for blood) using "Off-board Lysis" reagent settings.
  Recommendation(s): Add 100 µL of sample to 1000 µL of pre-aliquoted easyMAG lysis buffer (total input sample volume is 1100 µL). Incubate for 10 minutes at room temperature. Elution volume is 100 µL.
- **bioMérieux EMAG Instrument**
  Protocol: Custom protocol: **CDC Flu V1** using "Off-board Lysis" reagent settings.
  Recommendation(s): Add 100 µL of samples to 2000 µL of pre-aliquoted easyMAG lysis buffer (total input sample volume is 2100 µL). Incubate for 10 minutes at room temperature. Elution volume is 100 µL. The custom protocol, **CDC Flu V1**, is programmed on the bioMérieux EMAG instrument with the assistance of a bioMérieux service representative. Installation verification is documented at the time of installation. Laboratories are recommended to retain a record of the step-by-step verification of the bioMérieux custom protocol installation procedure.

PCR INSTRUMENTS

- Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4
- Applied Biosystems™ QuantStudio™ Dx with version 1.0.3 software
- QIAGEN Rotor-Gene Q MDx with AssayManager version 1.0.4.1 and Epsilon version 1.0.1 software

PCR REAGENTS AND CONTROLS

RT-qPCR ASSAYS

ePlex SARS-CoV-2 Test (GenMark Diagnostics, Inc.) ⌄

Simplexa COVID-19 Direct (DiaSorin Molecular LLC) ⌄

Abbott Real*Time* SARS-CoV-2 assay (Abbott Molecular) ⌄

Quest SARS-CoV-2 rRT-PCR (Quest Diagnostics Infectious Disease, Inc.) ⌄

Lyra SARS-CoV-2 Assay (Quidel Corp.) ⌄

COVID-19 RT-PCR Test (Laboratory Corporation of America) ⌄

Panther Fusion SARS-CoV-2 (Hologic, Inc.) ⌄

TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Inc.) ⌄

cobas SARS-CoV-2 (Roche Molecular Systems, Inc.) ⌄

New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel (Wadsworth Center, NYSDOH) ⌄

CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC) ⌄

OTHER ASSAYS & CONTROLS

- Obtaining N1/N2 Positive Controls, for the CDC EUA design:
    - Novel Coronavirus extracted RNA is available from BEI. To create N1/N2 positive controls from BEI's concentrated RNA, dilute the concentrated RNA into extracted nucleic acid to approximately 2 to 3 times the assay LOD per reaction. or
    - IDT sells a plasmid control (2019-nCoV_N_Positive Control #10006625). To create N1/N2 positive controls from IDT's plasmid control, dilute the plasmid into extracted nucleic acid to approximately 2 to 3 times the assay LOD per reaction.
- Obtaining RNase P (RP) Control, for the CDC EUA design:
    - Human RNA can be extracted from human specimens or cultured human cells and used directly as the RP positive control or
    - IDT sells a plasmid control (Hs_RPP30 Positive Control #10006626). Dilute the plasmid into extracted nucleic acid to approximately 2 to 3 times the assay LOD per reaction.

<u>SARS-COV-2 TESTS</u>

INTEGRATED DNA TECHNOLOGIES (IDT)
CDC Emergency Use Authorization Kits

CDC EUA kit includes all published assays in individual tubes which are premixed to the CDC's recommended working concentration and delivered in IDTE (1X TE buffer) pH 7.5. Shipped at ambient temperature. Orders of these kits are fulfilled from batches of material that have been QC qualified and released for sale under the Centers for Disease Control EUA. Estimated shipment is dependent on batch verification and release from CDC.

Quantity	Product	Catalog #	Price
	2019-nCoV CDC EUA Kit, 500 rxn	10006606	$125.00 USD

Updated March 20, 2020

The CDC has quality controlled and authorized primers and probes for the N1, N2 and RP assays from following batches to be sold under the CDC EUA (dated February 29, 2020, and as amended):

0000500383, 0000504847, 0000505594, 0000505969, 0000507295, 0000507509, 0000508150, and 0000508785

The CDC did not QC the N3 assays, and the N3 assay is not part of the authorized kits.   The FDA approved the CDC's amended protocol that excludes the N3 Assay on March 15, 2020.   All kits from all future batches of IDT primers and probes will <u>not</u> include the N3 assay.

Bioresearch Technologies

The following CDC-qualified lots of 2019-nCoV CDC Probe and Primer Kits are available:
143503, 143764

Catalog #	Item name	Price	Size
KIT-NCOV-PP1-1000	2019-nCoV CDC-qualified Probe and Primer Kits for SARS-CoV-2	$230	1000 rxns

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Source and additional information: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd

DEFINING THE SEROLOGICAL EPIDEMIOLOGY OF COVID19 AT MD ANDERSON

OUTLINE
RESEARCH WORKING GROUP MEMBERS
KEY POINTS
SUMMARY
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PROPOSAL FOR SEROLOGY TESTING

WORKING GROUP
DEVELOPMENT OF AN ELISA FOR COVID19 SEROLOGIC EPIDEMIOLOGY

Nadim Ajami, PhD
Eleonora Dondossola, PhD
Jennifer Wargo, MD
Raghu Kalluri, MD
Cassian Yee, MD
Dongxing Zha, PhD
Jason Bock, PhD

KEY POINTS
- The high rate of asymptomatic SARS-CoV-2 infections (estimated up to 88%) presents a challenge to our understanding of COVID19 epidemiology.
- The development of a serologic test (ELISA) to detect circulating antibodies against SARS-CoV-2 is imperative to understand seroprevalence rates in asymptomatic and mild cases as well as the duration of the immune response. This is critical to fight against COVID19 by identifying individuals who have overcome the infection and are presumed immune.
- Learnings from recent human and animal studies to related to COVID19 and others from previous coronavirus outbreaks (i.e. SARS and MERS) show that natural infection can lead to protective immunity, and in the case of SARS and MERS, such immunity lasts up to three years.
- There are quantitative serologic tests that are currently available commercially, however the only FDA-approved serologic test to date is not quantitative. Nonetheless, it is important to capitalize on available data to help our workforce and eventually our patients.
- By implementing serology testing we will be able to determine the rate of seroprevalence in our community which will help determine who can safely return to work and care for our patient population.

SUMMARY
A serologic test for COVID19 detects the presence and measures the amount and type of antibodies in the blood elicited after the infection, irrespective of the presence of clinical symptoms. Although this test has limitations in diagnosing active COVID19, it plays a critical role in the fight against it by identifying individuals who have overcome the infection and are presumed immune even in asymptomatic cases which have been reported as high as 88%[1]. This test will help us determine which individuals have been exposed to the SARS-CoV-19, the causing agent of COVID19, and are presumed protected from reinfection. Validated serologic tests will be crucial for epidemiologic studies and to help shape decisions of who can could safely return to work.

Although few information is available for SARS-CoV-2, protective immunity has been shown in primates after re-challenge to CoV2, with no evidence of viral loads and no viral replication in all primary tissue compartments at five days post-reinfection[2].

Seroconversion (IgG and IgM) in SARS-CoV-2 has been shown to occur by 7 days in 50% of patients and 14 days in all of them, as reported[3] (Fig 1[3]). Similar results were confirmed by an independent group[4]. A more comprehensive study (n=175) detected neutralizing antibodies in >95% of patients positive to CoV2 using a neutralization assay[5].

There are many formats in which serologic tests can be carried out but in this case an enzyme-linked immunosorbent assay (ELISA) is the most optimal option. ELISAs provide a relatively quick (24 hours turnaround time) and inexpensive readout of antigen-antibody interaction that can be quantitative and specific for viral antigens and human antibodies. Previous work with SARS and MERS outbreaks, both caused by coronaviruses, identified an ELISA method that provided high sensitivity to detect the virus and was more efficient than other approaches. No cross-reactivity was shown to other coronaviruses (OC43, HKU1, 229E, NL63) in ELISA assays recently developed to test SARS-CoV-2[4,6,7].

Fig. 1 | Viral load kinetics, seroconversion and clinical observations in individual cases. Panels A to I correspond to cases #1, #2, #3, #4, #7, #8, #10, #14, and #16. Dotted lines, limit of quantificationwere performed in duplicate and the data presented are means of results. Experiment obtained by two laboratories independently.

As other institutions have noted so far, epidemiologic studies are urgently needed to help uncover the burden of disease, in particular the rate of asymptomatic infections, and to get better estimates on illness and death. These epidemiologic studies can help identify the extent of virus spread in households, communities, and specific settings like our hospital and research buildings, which could help guide control measures. In the near future, serologic assays will be needed for evaluation of results of vaccine trials.

To date, only one assay has been approved by the FDA (Cellex, lateral flow assay) to detect circulating antibodies against SARS-CoV-2, but this assay does not offer a quantitative readout nor can it distinguish the type of antibody measured. Given the lack of available solutions, it is imperative that our institution invests in developing a serologic test to be used for preclinical purposes with the potential of implementation in the CLIA laboratory. To this end, we have engaged with external groups (Florian Krammer, Mount Sinai and Tony Piedra, Baylor College of Medicine) to source reagents and support collaborators in the development of serologic tests. In addition, reagents and methodologies have been developed at our institution (Dr. Raghu Kalluri) prior to the halt on research activities that can be used to produce a serologic test. These assays will be developed in non-CLIA environments but SOPs will be created for an eventual transition to MD Anderson's CLIA lab if needed. The serologic epidemiology studies will be carried out by selecting the most optimal assay based on sensitivity and specificity values together with ease of reagent availability, throughput, and assay implementation.

To give us the best chance of success we propose to develop two related, but distinct ELISAs. One based on binding to just the receptor binding domain (RBD) of the Spike protein (BCM) and one based on the full length Spike protein (MDA). The Baylor group has been operating for several weeks and is somewhat ahead of the MDA group, but we will have more control over the development, characterization and utilization of the MDA developed assay.

WORK GROUP: Drs. Nadim Ajami, Eleonora Dondossola, MDA; Tony Piedra, Baylor College of Medicine; Florian Krammer, Mount Sinai

Goal: Develop a quantitative ELISA to detect IgM and IgG in human plasma/serum against SARS-CoV-2 spike protein and the receptor binding domain (**RBD ELISA**)

Reagents: Proteins and plasmids expressing SARS-CoV-2 spike protein and RBD have been shared by Dr. Florian Krammer (Icahn School of Medicine, Mount Sinai; several in-house tests have been developed worldwide based on these reagents, including multiple Stanford labs); and shared with Dr. Piedra at BCM to develop an ELISA under RUO settings and to be later transferred to a CLIA environment. 12.5 µl of plasma or serum needed/test (2 wells, in duplicate, 6.25 µl/well)

Results: Krammer's antigen was successfully recognized by anti-CoV2 antibodies, while commercially available antigens were not (possibly due to production in mammalian cells that support better folding and post-translational modifications), see table below. The BCM group will start testing sera from symptomatic cases and asymptomatic individuals this week.

		OD values of SARS CoV-2 ELISA								
		mAb/polyclonal/human serum								
	Subunit	Mouse CoV-1-S1 (ThermoFisher)	Rabbit CoV-2-S1 (SinoBiological)	Rabbit CoV-1-S (FDA)	Rabbit CoV-2-S (FDA)	Rabbit Neg control serum (FDA)	Flor (human serum)	PAP (human serum)	204490769 (human serum)	204490775 (human serum)
SARS-CoV-2	S1 (230-01101,RayBiotech)	0.3,0.2	0.3,0.1	0.1,0.1	0.1,0.1	0.1,0.1	1.3,1.7	1.8,2.0	1.3	1.1
	RBD (230-01102,RayBiotech)	0.2,0.2	0.2,0.1	0.1,0.1	0.1,0.1	0.1,0.1	3.1(1:2) 1.7,2.0	1.8,1.9	1	0.7
	RBD (Florian Krammer)	0.1,0.1	>4.0,>4.0	0.1,0.1	3.8,>4.0	0.1,0.1	0.2 0.2,0.2	0.3,0.2	0.1	0.1

WORK GROUP: Raghu Kalluri, ORBIT, Cassian Yee

Goal: Develop an ELISA to detect IgM and IgG in human plasma/serum against SARS-CoV-2 spike protein (**SPIKE ELISA**)

Reagents: Recombinant soluble SARS-CoV-2 Spike ectodomain protein production in 293T cells was achieved before research activities were suspended. More protein and other reagents can be produced but this will require clearance to return to the laboratory to resume the work. ORBIT will perform QC testing to release batches of Spike protein and ensure consistency. Yee lab will develop neutralization assays on pseudovirus to safely determine relevance of positive serum to immunity.

RESEARCH PROPOSAL FOR TESTING OUR WORKFORCE

SEROLOGICAL EPIDEMIOLOGY FOR COVID19 AT MD ANDERSON CANCER CENTER

PHASE I

1. Reagent Development & Assay Validation

· *Activities:* Produce recombinant RBD to keep on performing RBD ELISA; produce recombinant Spike protein to perform SPIKE ELISA; include a robust QC panel to use for internal development and share with collaborators; develop and qualify a serological test using a limited (<10 de-identified patient samples with confirmed molecular positive and negative for CoV-19). Development of neutralization assay.

· *Timeline:* 4 weeks
· *Resources*: 4 FTEs (3 individuals for production, testing and data reporting and 1 supervisor); 1 FTE for QC testing; 2 FTEs for Neutralizing assay development

· *Equipment*: ELISA plate readers are available
· *Estimated cost and supplies*: Cost of routine lab supplies to be covered by participating labs

PHASE II

2. Assay Utilization
· *Activities:* Run an initial epidemiologic study on presumed cases based on symptomatology but without a positive COVID19 test and 500 employees from MDACC research (~20%) modeled after the national NAIAD study and include a questionnaire to determine prior symptoms. This would help us understand the existing exposure level for our employees and help quantify the risks of opening to future exposures. This would be run by research personnel on research staff, with no impact on the clinic
· *Timeline:* start in 4 weeks, in course as permitted
· *Effort:* 7 FTEs

PHASE III

3. Testing Expansion
Based on the results of Phase I and the then current landscape, determine if we should extend the testing to the wider research community as an input into a staged, risk-based, informed, back-to-plan for research.
· *Timeline*: *to be determined*

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	Subunit	Mouse CoV-1-S1 (ThermoFisher)	Rabbit CoV-2-S1 (SinoBiological)	Rabbit CoV-1-S (FDA)	Rabbit CoV-2-S (FDA)	Rabbit Neg control serum (FDA)	Flor (human serum)	PAP (human serum)	204490769 (human serum)	204490775 (human serum)
SARS-CoV-2	S1 (230-01101,RayBiotech)	0.3,0.2	0.3,0.1	0.1,0.1	0.1,0.1	0.1,0.1	1.3,1.7	1.8,2.0	1.3	1.1
	RBD (230-01102,RayBiotech)	0.2,0.2	0.2,0.1	0.1,0.1	0.1,0.1	0.1,0.1	3.1(1:2) 1.7,2.0	1.8,1.9	1	0.7
	RBD (Florian Krammer)	0.1,0.1	>4.0,>4.0	0.1,0.1	3.8,>4.0	0.1,0.1	0.2 0.2,0.2	0.3,0.2	0.1	0.1

WORK GROUP: Raghu Kalluri, ORBIT, Cassian Yee

Goal: Develop an ELISA to detect IgM and IgG in human plasma/serum against SARS-CoV-2 spike protein (**SPIKE ELISA**)

Reagents: Recombinant soluble SARS-CoV-2 Spike ectodomain protein production in 293T cells was achieved before research activities were suspended. More protein and other reagents can be produced but this will require clearance to return to the laboratory to resume the work. ORBIT will perform QC testing to release batches of Spike protein and ensure consistency. Yee lab will develop neutralization assays on pseudovirus to safely determine relevance of positive serum to immunity.

RESEARCH PROPOSAL FOR TESTING OUR WORKFORCE

SEROLOGICAL EPIDEMIOLOGY FOR COVID19 AT MD ANDERSON CANCER CENTER		
PHASE I	**PHASE II**	**PHASE III**
ASSAY DEVELOPMENT *4 weeks*	**ASSAY UTILIZATION** *Start in 4 weeks, in course as permitted*	**ASSAY EXPANSION** *To be determined*

BCM/MDA – ELISA
- Quantitative
- Antigen: Receptor Binding Domain (RBD)
- Antibody: IgG and IgM
- Controls: Positive sera and mAb/polyclonal sera

MDA – ELISA
- Quantitative
- Antigen: Spike protein (full-length)
- Antibody: IgG and IgM
- Controls: Positive sera and mAb/polyclonal sera

ASSAY SELECTION
One assay will be selected based on sensitivity, specificity, and ease of implementation.

SET 1
500 Research, healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

VALIDATION SAMPLES
~10 Residual samples from patients recovered from COVID19

COVID19 ELISA

Positive → Presumed Immune

Negative → Not presumed immune

IgM + ; IgG - Resolving infection
IgM + ; IgG + Convalescent
IgM - ; IgG + Recovered

- Expansion to wider population
- Repeat testing to determine duration of antibodies in circulation
- Development of neutralization assays

PHASE I

1. Reagent Development & Assay Validation

· *Activities:* Produce recombinant RBD to keep on performing RBD ELISA; produce recombinant Spike protein to perform SPIKE ELISA; include a robust QC panel to use for internal development and share with collaborators; develop and qualify a serological test using a limited (<10 de-identified patient samples with confirmed molecular positive and negative for CoV-19). Development of neutralization assay.

· *Timeline:* 4 weeks
· *Resources:* 4 FTEs (3 individuals for production, testing and data reporting and 1 supervisor); 1 FTE for QC testing; 2 FTEs for Neutralizing assay development

· *Equipment:* ELISA plate readers are available
· *Estimated cost and supplies:* Cost of routine lab supplies to be covered by participating labs

PHASE II

2. Assay Utilization
· *Activities:* Run an initial epidemiologic study on presumed cases based on symptomatology but without a positive COVID19 test and 500 employees from MDACC research (~20%) modeled after the national NAIAD study and include a questionnaire to determine prior symptoms. This would help us understand the existing exposure level for our employees and help quantify the risks of opening to future exposures. This would be run by research personnel on research staff, with no impact on the clinic
· *Timeline:* start in 4 weeks, in course as permitted
· *Effort:* 7 FTEs

PHASE III

3. Testing Expansion
Based on the results of Phase I and the then current landscape, determine if we should extend the testing to the wider research community as an input into a staged, risk-based, informed, back-to-plan for research.
· *Timeline:* *to be determined*

REFERENCES

1.	Sutton D, Fuchs K, D'Alton M, Goffman D. Universal Screening for SARS-CoV-2 in Women Admitted for Delivery. *New England Journal of Medicine* 2020.
2.	Bao L, Deng W, Gao H, et al. Reinfection could not occur in SARS-CoV-2 infected rhesus macaques. *bioRxiv* 2020: 2020.03.13.990226.
3.	Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020.
4.	Okba NMA MM, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe Acute Respiratory Syndrome Coronavirus 2–Specific Antibody Responses in Coronavirus Disease 2019 Patients: Centers for Disease Control and Prevention, 2020.
5.	Wu F, Wang A, Liu M, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. *medRxiv* 2020: 2020.03.30.20047365.
6.	Amanat F, Nguyen T, Chromikova V, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *medRxiv* 2020: 2020.03.17.20037713.
7.	Guo L, Ren L, Yang S, et al. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). *Clinical Infectious Diseases* 2020.

TITLE: Evaluating the seroprevalence of SARS-CoV02 antibodies in an asymptomatic or subclinical population at MD Anderson Cancer Center

Study Chair: Jeniffer Wargo
Co-Chairs: Eleonora Dondossola, Nadim Ajami
Collaborators: Florian Krammer, Pedro Piedra
Department: Genomic Medicine
Phone:
Unit:

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PROTOCOL APPLICATION

Participant Population

Expected age range of participants:
18 and above

Will this include embryonic stem cells?
No

Does this research include MDACC employees as participants?
Yes

Total expected number of participants is 500-10,000 COVID19 patients who undergo XX from XX day through day.

Specimen Type and Collection Type:
Residual blood serum samples

Total # of Specimens, Volume, Frequency:

1 sample per patient or as many as avaialble

List the source of specimens/data (Select all that apply):
The data of patients will be collected from the electronic medical record (Epic).

Data Profile

Types of data points that will be collected include:
Names or initials, medical record number, date of birth, gender, medications, medical comorbidities, smoking history, dates of surgery and other treatments at MD Anderson including intravenous or oral antibiotics administered and/or prescribed, dates of followup, results of imaging/pathology/labwork, date of recurrence/progression, and date of death.

Specimens/data sharing
Specimens/data will NOT be shared with any entity, person, or organization outside of MD Anderson

Confidentiality

Will there be a link to identify subjects?
YES-PHI will be collected however only made available to the PI and mid-level providers/research/clinical fellow participating in this research study

Will identifiable data be made available to anyone other than the PI?
YES-Research/clinical fellow and mid-level providers participating in this research study

Compensation
Will subjects receive compensation (e.g. water bottles, t shirts, gift cards), or reimbursements (e.g., gas cards for travel, parking vouchers, bus fare, or hotel reimbursement?)
No

Informed Consent Considerations

Please select the appropriate response(s) regarding the informed consent process for this study (you may select more than one)
Protocol staff will obtain prospective written consent

1.0 Objectives

The **primary aim** of this research study is to **determine the seroprevalence of antibodies against SARS-CoV-2, the causing agent of COVID19**. This study represents the initial step to determine the rate of seroposivitiy among our workforce. The data resulting from this study will be used to guide the development of a larger study.

Outcome Measures:
Primary:
- Prevalence of total antibodies against the receptor binding domain of the spike proteins of SARS-CoV-2

Secondary:
- Prevalence of IgG and IgM antibodies against surface antigens of SARS-CoV-2
- Isotyping of antibodies against surface antigens of SARS-CoV-2

2.0 Rationale

Severe acute respiratory disease coronavirus 2 (SARS-CoV-2) has caused a pandemic known as coronavirus disease 2019 (COVID-19). Patient's symptoms range from no detectable signs of infection, to fever, cough, acute respiratory distress syndrome and pneumonia that can lead to death in 1.4% of cases (ref). Several molecular assays to directly detect viral RNA through real time reverse transcriptase-PCR are currently available to identify positive patients3,4. However, these tests have to be performed in a very specific time frame during the infection to avoid false negative. Testing of asymptomatic people is thus challenging and often does not occur due to lack of resources and prioritization of symptomatic people, leaving many undiagnosed.

A serologic test for COVID19 detects the presence and measures the amount and type of antibodies in the blood elicited after the infection, irrespective of the absence of clinical symptoms (which have been reported as high as 88%1) and would complement PCR-based approaches, identifying individuals who have overcome the infection and are presumed immune. Although limited information is available for SARS-CoV-2, protective immunity has been shown in primates after re-challenge to CoV2, with no evidence of viral loads and no viral replication in all primary tissue compartments at five days post-reinfection (Fig. 1) (ref).

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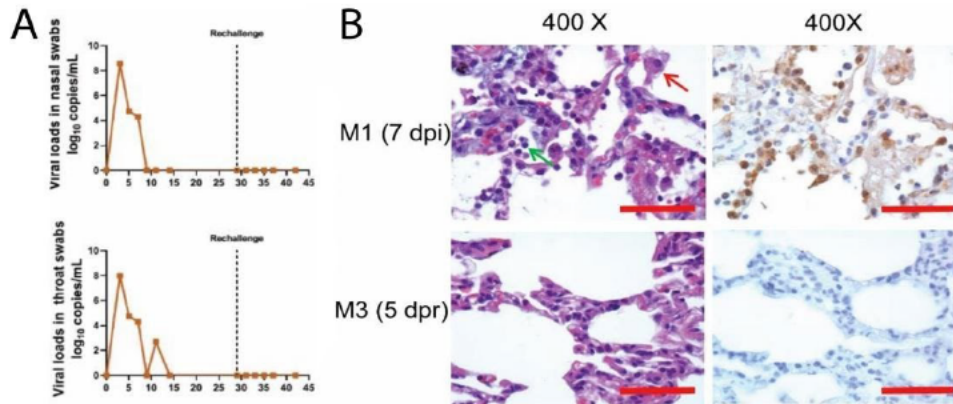
Figure 1. Lung exhibited moderate interstitial pneumonia with infiltration of lymphocytes (green arrow) and swollen aveolar macrophages (red arrow) in the alveolar cavities and positivity to anti-Spike antibody.

Seroconversion (IgG and IgM) in SARS-CoV-2 has been shown to occur by 7 days in 50% of patients and 14 days in all of them, as reported (ref) (Fig. 2(ref)). Similar results were confirmed by an independent group (ref).

A more comprehensive study (n=175) detected neutralizing antibodies in >95% of patients positive to CoV2 using a neutralization assay (Fig. 3) (ref)

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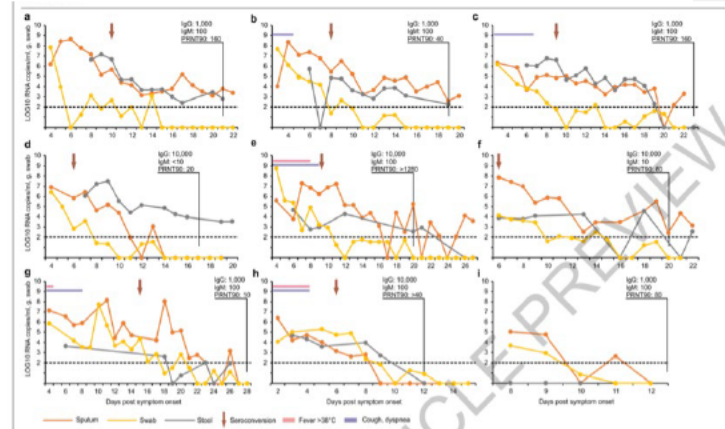
Figure 2. Viral load kinetics, seroconverstion, and clinical observations in individual cases. Panels A to I correspond to 9 different cases studies. Dotted line, limit of quantification were performed in ducplicate and the data presented are means of

There are many formats in which serologic tests can be carried out but in this case an enzyme-linked immunosorbent assay (ELISA) is the most optimal option. ELISAs provide a relatively quick (24 hours turnaround time) and inexpensive readout of antigen-antibody interaction that can be
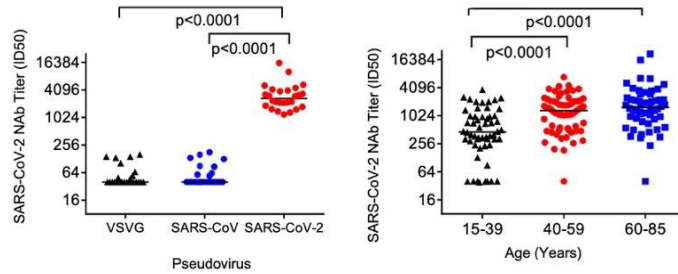
Figure 3.

quantitative and specific for viral antigens and human antibodies. Previous work with SARS and MERS outbreaks, both caused by coronaviruses, identified an ELISA method that provided high sensitivity to detect the virus and was more efficient than other approaches. No cross-reactivity was shown to other coronaviruses (OC43, HKU1, 229E, NL63) in ELISA assays recently developed to test SARS-CoV-2 (Fig. 4) (refs).
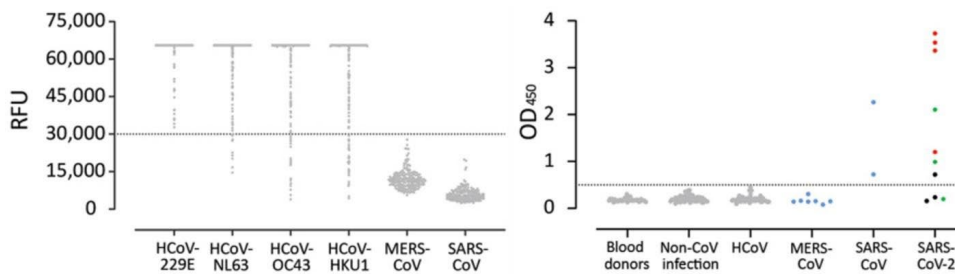
Figure 4.

Epidemiologic studies are urgently needed to help uncover the burden of disease, in particular the rate of asymptomatic infections, and to get better estimates on illness and death. These epidemiologic studies can help identify the extent of virus spread in households, communities, and specific settings like our hospital and research buildings, which could help guide control measures. In the near future, serologic assays will be needed for evaluation of results of vaccine trials.

Hypothesis: we hypothesize that SARS-CoV-2 induces seroconversion in exposed individuals, stimulating the production of circulating IgG and IgM antibodies.

3.0 Eligibility of Subjects

Inclusion: Adult subjects (≥18 years) with a laboratory confirmed diagnosis of COVID19 or under investigation for possible COVID19:

- Residual blood serum samples from laboratory confirmed COVID19 cases or from presumed cases (persons under investigation) will be obtained from the Institutional Tissue Bank (ITB) at MD Anderson Cancer Center

Exclusion: A subject will not be eligible for inclusion in this study if any of the following criteria apply:

- Samples kept in suboptimal conditions will be excluded from this study.

4.0 Research Plan and Methods

4.1 Subjects

Blood serum residual samples obtained from the Institutional Tissue Bank from laboratory confirmed COVID19 cases and persons under investigation (PUIs) will be assayed to determine the presence and quantity of SARS-CoV-2 antibodies against the viral receptor binding domain (RBD)of the spike protein.

4.2 Sample Collection

Blood samples

Potential Harms/Adverse events:

Safety Monitoring:

4.3 Testing

The immunoassay that will be used to study the seroprevalence of antibodies against SARS-CoV-2 is being developed in collaboration with Florian Krammer, Professor of Vaccinology at the Department of Microbiology at the Icahn School of Medicine at Mount Sinai, the Principal Investigator of the Sinai-Emory Multi-Institutional Influenza Vaccine Innovation Center (SEM-CIVIC), and member of the NIH-funded Centers for Excellence in Influenza Research and Surveillance (CEIRS). Dr Krammer has shared reagents and a protocol for the development of the assay and such reagents are being shared with Dr. Pedro Piedra at Baylor College of Medicine who is leading the implementation of Dr. Krammers' protocol. Dr. Piedra is a Professor in the Department of Molecular Virology and Microbiology and his research is focused on influenza virus,

respiratory syncytial virus, adenovirus viruses, human metapneumovirus, and the recently described SARS-CoV-2.

This enzyme-linked immunosorbent assay (ELISAs) is based on recombinant antigens derived from the sp ke protein of CoV-2, including its receptor binding domain (RBD). Such antigen was used for testing 59 negative human control sera preCOVID-19, including people with previous confirmed coronavirus exposure (e.g. NL63) and four samples from CoV-2positive patients. ELISAs proved sensitive and specific and allowed for identification of positive patients using human plasma/serum as early as three days post-symptom onset (12.5 µl of plasma or serum needed/test; 2 wells, in duplicate, 6.25 µl/well). As an advantage, these assays do not require handling of infectious virus, are amenable to scaling and only few microliters of plasma/serum are needed.

5.0 Statistics and Justification of Sample Size

Sample size justification

Data analysis

6.0 Procedure to Obtain Informed Consent

7.0 Data Confidentiality

Data will be available to the PI and people directly involved with the collection and analysis of data related to this project. IRB approval will be obtained for any exchange of data within and outside of MD Anderson.

Collection of Identifiers:
Identifiers (name, dates, phone number, email address and MRN) will be collected. Identifiers (other than dates) will be replaced by study numbers in the analytic file. The key linking these numbers will be retained in a locked file or stored on an encrypted server behind the firewall, by the investigator's designated personnel. Dates will be retained as a limited data set. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval.

Training of Personnel:
All MD Anderson personnel will be fully trained to maintain the patient health information confidentially. Training will be documented as required by institutional policy.

Data Storage:
The PI and research staff will attempt to minimize risk through only storing information containing subject identifiers in locked file storage, and on password-protected computers, on encrypted servers behind an institutional firewall and according to current institutional and federal data security requirements. In addition, access to patient identifiers will be limited to the minimum number of necessary research personnel, and only to those research personnel directly involved with obtaining patient information and assigning random study identifiers. Keys containing information linking study subjects to personal identifiers will be maintained in locked storage for paper records or behind institutionally approved firewall and electronic security measures for electronic keys, and available ONLY to the PI and research personnel directly involved in creating random study identifiers. Information containing subject personal identifiers will not be removed from MD Anderson Cancer Center without IRB approval and will not be shared in publications or reports concerning this research study.

Data Sharing:
Study data will not be shared with any individuals or entities that are not involved in the study. No identifying information will be shared with outside collaborating sites or outside collaborating research staff without prior IRB approval and a data use or material transfer agreement has been implemented. Sharing of data will be done after approval of the PI and only by secure mechanisms, as approved by MD Anderson Information Security.

Final Disposition of Study Records:
These data will be used for this research study. Data that is in hard-copy form will be retained on site until the study is terminated, and may be stored indefinitely, per institutional standards, in long-term off-site storage with an MD Anderson approved, secured contract site. Electronic data will be retained indefinitely on MD Anderson servers behind the institutional firewall. Data will not be shared with any party outside of MD Anderson and will not be retained or disseminated for other research without prior IRB approval. Study data and paper records will not be destroyed but will be retained permanently.

8.0 References

COVID19 ANTIBODY RAPID SCREENING ALGORITHM FOR PATIENTS, HEALTHCARE AND ESSENTIAL WORKERS INCLUDING THOSE IN HIGH-RISK AREAS NOT PRESENTING SYMPTOMS OF DISEASE

PRIORITY LEVEL 1

Healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

PRIORITY LEVEL 1

Patients recovered from COVID19

PRIORITY LEVEL 2

Patient-facing HCWs not presenting symptoms

PRIORITY LEVEL 3

Essential workers and others in high-risk areas not presenting symptoms

PRIORITY LEVEL 3

Blood products

Serology → **Negative** → Not presumed immune → Retest in 45 days in the absence of symptoms (N/A for blood products)

Positive

RT-qPCR (MolDx Lab) → **Negative** → Serology → **Negative** → Retest in 21 days → **Negative** → Not presumed immune

Positive

Isolation

Retest in 14 days

If prior serology test was positive

Positive

Presumed Immune

IN-HOUSE TESTING: PRIORITY LEVELS 1 & 2
SEND-OUT: PRIORITY LEVELS 2 &3

A detailed protocol for a serological assay to detect SARS-CoV-2 seroconversion in humans: antigen production and test setup

Daniel Stadlbauer[1#], Fatima Amanat[1,2#], Veronika Chromikova[1], Kaijun Jiang[1], Shirin Strohmeier[1,3], Guha Asthagiri Arunkumar[1,2], Jessica Tan[1,2], Disha Bhavsar[1], Christina Capuano[1], Ericka Kirkpatrick[1,2], Philip Meade[1,2], Nichalle Britto[1], Catherine Teo[1], Meagan McMahon[1], Viviana Simon[1,4,5], Florian Krammer[1]*

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[4]Global Health Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, NY, USA
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#contributed equally

Abstract

In late 2019, cases of atypical pneumonia were detected in China. The etiological agent was quickly identified as betacoronavirus (named SARS-CoV-2) which has since caused a pandemic. Several methods allowing for the specific detection of viral nucleic acids have been established but only allow detection of the virus during a short period of time, generally during acute infection. Serological assays are urgently needed to conduct serosurveys, to understand the antibody responses mounted in response to the virus and last but not least for identifying individuals who are potentially immune re-infection. Here we describe a detailed protocol for expression of antigens derived from the spike protein of SARS-CoV-2 that can serve as substrate for immunological assays as well as a two-step serological enzyme-linked immunosorbent assay (ELISA). These assays can be used for research studies as well as for testing in clinical laboratories.

48
49   **Introduction**
50
51   Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes
52   **CO**rona**VI**rus **D**isease 2019 (COVID19), emerged in late 2019 in Wuhan, China[1,2]. The virus
53   rapidly spread globally causing a pandemic. Currently, no drugs or antivirals are available and
54   countermeasures are limited to non-pharmaceutical interventions (NPIs). Nucleic acid-based
55   tests for detection of the virus during acute disease are in use worldwide[3,4].
56   However, the development of serological assays has been lagging behind due to lack of suitable
57   reagents. Serological assays are needed to perform serosurveys aimed at determining the real
58   infection rate and infection fatality rate in a given population. Furthermore, they are useful to
59   characterize the immune response to the virus in a detailed qualitative and quantitative manner.
60   In addition, serological assays are also of immediate practical use. They can be used to identity
61   individuals who were infected (including severe, mild and asymptomatic cases) and who are now
62   potentially immune. A recent study in non-human primates showed that reinfection, at least in the
63   small number of animals used in this study, does not occur[5] once antibody responses have been
64   mounted. Infection with coronaviruses circulating in human populations such as HKU, NL63 etc.
65   also leads to immunity that protects from re-infection for months to years[6]. Therefore, individuals

Figure 1: Protocol overview.
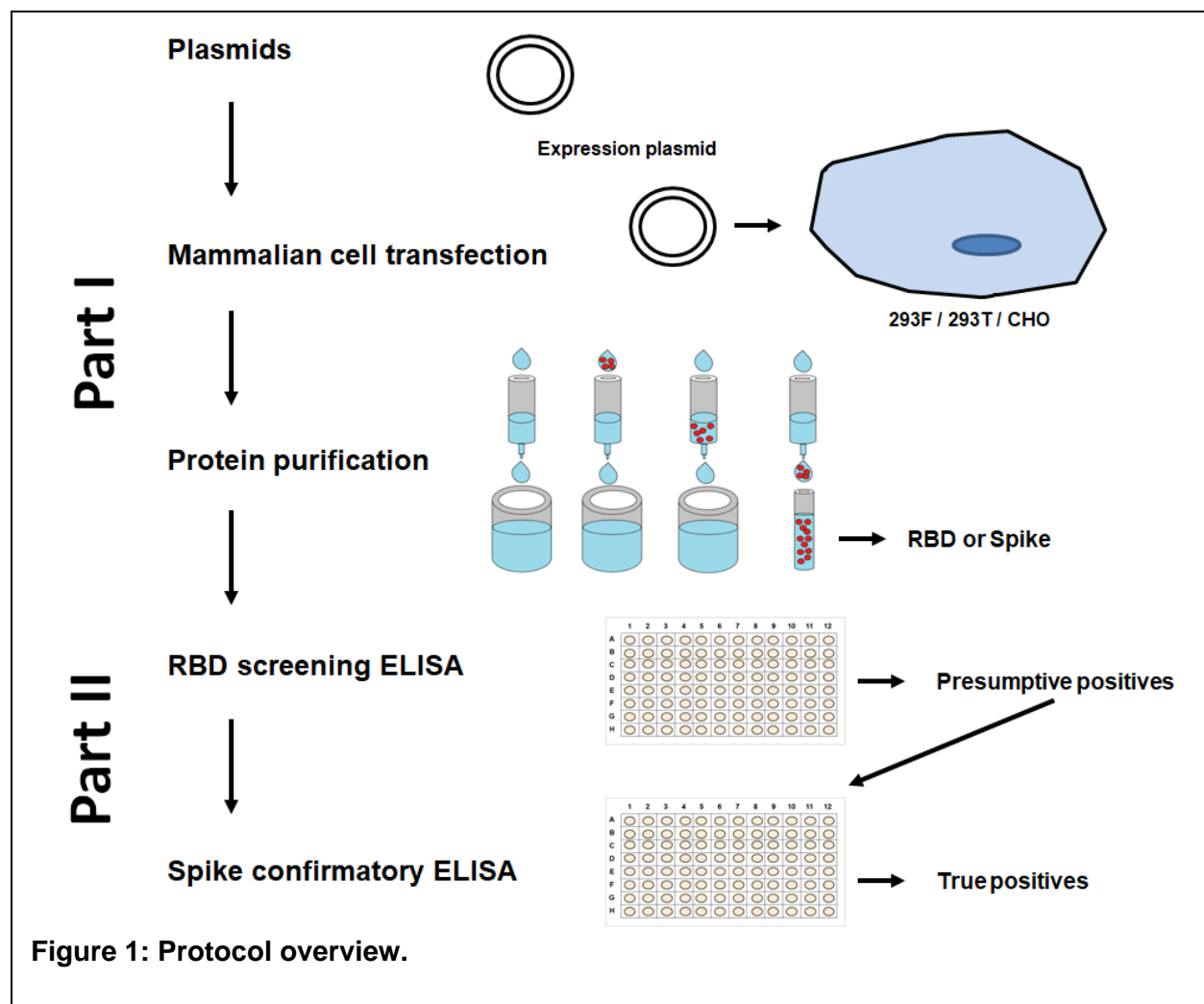
66 who have mounted an immune response to SARS-CoV-2 are likely immune, which means that
67 they are unlikely to transmit the virus to others. As an example, health care workers who are
68 immune can take care of COVID19 patients with minimal risk to themselves, their colleagues and
69 other patients. In addition, the use of convalescent serum may serve as valuable treatment option
70 for patients with severe COVID19, especially in the absence of other options. A serological assay
71 is critical for identifying potential blood donors.
72 The surface glycoprotein of the virus, termed spike (S) protein, mediates attachment of the virus
73 to human cells via its receptor binding domain (RBD)[7] and mediates fusion of viral and cellular
74 membranes. Antibodies that bind to the spike protein, and especially to the RBD domain, can
75 neutralize coronaviruses. We used, therefore, different recombinant spike protein preparations as
76 antigen for our ELISA. We reported in our in earlier work that individuals not exposed to SARS-
77 CoV-2 are completely naïve for the spike protein and show no reactivity in an ELISA[8]. It is,
78 therefore, easy to distinguish between exposed/immune and naïve people.
79 In this report, we provide detailed protocols for expressing the needed antigen(s) (**Part I**) as well
80 as for setting up the ELISA that we have developed (**Part II**) (**Figure 1**). We believe that this
81 protocol will be useful not only for research laboratories around the globe but also for testing in
82 diagnostic/clinical laboratories. The described protocol setup works well for us but it can easily be
83 modified, adapted to local needs and improved by the research community in the future.
84 Mammalian expression plasmids for the generation of the recombinant proteins are available from
85 the corresponding author.
86
87 **Part I: Mammalian cell transfection and protein purification protocol**
88
89 This protocol can be used for both expression vectors for the secreted RBD as well as a soluble
90 trimeric version of the SARS-CoV-2 spike protein. Expression levels of the RBD are very high in
91 our hands (>20 mg/l culture) while expression levels for the full length spike are lower
92 (approximately 1 mg/ml). Therefore, we use the recombinant RBD for initial screening ELISAs
93 and the full length spike for confirmatory ELISAs (as described in **Part II**). Preparation of plasmids
94 for mammalian cell expression is not described here. The plasmids all carry a betalactamase
95 (amp) resistance gene. They are grown in *E. coli* at 37°C (or 30°C) in shaker flasks over night.
96 High quality plasmid DNA can be obtained using commercially available maxiprep kits (ideally
97 with an endotoxin removal step). Importantly, other cell lines (293T, CHO etc.), other media,
98 transfection reagents and more sophisticated protein purification methods might be used as
99 alternatives. Of note, cells can also be transfected in regular flasks in regular incubators without
100 shaking.
101
102 **MATERIALS**
103   ● Expi293 Expression Medium (Gibco #A1435102)
104   ● Opti-MEM™ I Reduced Serum Medium (Gibco #31985088)
105   ● ExpiFectamine™ 293 Transfection Kit (GIbco #A14524)
106   ● PBS (1X) (Gibco #10010-023 or equivalent)
107   ● Ni-NTA Agarose (Qiagen #30230 or equivalent)
108   ● SDS-PAGE gels (Bio-Rad #4561094 or equivalent)
109   ● SDS-PAGE cell and power supply
110   ● Sodium phosphate monobasic monohydrate $NaH_2PO_4 . H_2O$ (Sigma Aldrich #S3522 or
111   equivalent)
112   ● Sodium Chloride NaCl (Sigma-Aldrich #S3014 or equivalent)
113   ● Imidazole (Sigma-Aldrich #I5513 or equivalent)

114   ● Disposable Polycarbonate Erlenmeyer Flasks (Corning #431147)
115   ● Trypan blue solution, 0.4 % (Gibco #15250-06 or equivalent)
116   ● Cell counting slides (Invitrogen #C10312 or equivalent)
117   ● 5mL Polypropylene columns (Qiagen #34964 or equivalent)
118   ● Amicon™ Ultra Centrifugal Filter Units 10 kDa (MilliporeSigma #UFC901024 or
119   equivalent)
120   ● Amicon™ Ultra Centrifugal Filter Units 50 kDa (MilliporeSigma #UFC905024 or
121   equivalent)
122   ● Polypropylene sterile conical tubes
123   ○ 15 mL (Denville Scientific #C1018P or equivalent)
124   ○ 50 mL (Fisher Denville Scientific #C1060P or equivalent)
125   ● Sterile, serological pipettes
126   ○ 5mL (Falcon #356543 or equivalent)
127   ○ 10mL (Falcon #357551 or equivalent)
128   ○ 25 mL (Falcon #357535 or equivalent)
129   ○ 50 mL (Falcon #356550 or equivalent)
130   ● Micropipette tips
131   ○ 20 µL barrier tips (Denville Scientific #P1121 or equivalent)
132   ○ 200 µL barrier tips (Denville Scientific #P1122 or equivalent)
133   ○ 200 µL tips (USA Scientific #1111-1700 or equivalent)
134   ○ 1000 µL barrier tips (Denville Scientific #P1126 or equivalent)
135   ● 1.5 mL Eppendorf tubes (Denville #C2170 or equivalent)
136   ● Stericup Quick Release-GP Sterile Vacuum Filtration System (MilliporeSigma
137   S2GPU05RE or equivalent)
138   ● Pipet-Aid
139   ● Micropipettes
140   ● Class II biological safety cabinet
141   ● Timer
142   ● Countess II cell counter or equivalent
143   ● CO_2 incubator with built in shaker (Eppendorf New Brunswick S41i or Equivalent)
144   ● Benchtop shaker (Benchmark #BT3000 or equivalent)
145   ● Cooling Centrifuge (Eppendorf 5810R or equivalent)
146   ● Refrigerator at 4°C (+/- 1°C)
147   ● Ultra-Low Freezer (-80°C)
148
149 **DEFINITIONS**
150
151   ● RBD = Receptor Binding Domain of SARS-CoV-2 (NR-52306)
152   ● PBS = Phosphate-Buffered Saline
153   ● RT = Room Temperature (18-25°C)
154   ● MEM = Minimum Essential Medium
155   ● DNA = Deoxyribonucleic Acid
156   ● Ni-NTA = Nickel-Nitrilotriacetic acid
157
158 **PROCEDURE:**
159
160 **Transfection in mammalian cells:**

4

161 HEK 293F cells are counted using an automated cell counter (or a regular counting chamber) and
162 seeded at a density of 600,000 cells/ml in Expi293 expression medium. The viability of the cells
163 must be greater than 90% at all times. Cells are passaged every 3-4 days and incubated in an
164 orbital shaking incubator at 37°C and 125 RPM with 8% CO2. A maximum cell density of 4-5 x
165 10^6 cells/ml is recommended and at this point, cells should be immediately passaged.

166 Transfections are performed according to manufacturer's instructions. 600 x 10^6 cells are
167 suspended in 200 ml of Expi293 expression media in a 1 L shaker flask. Twelve ml of Opti-MEM
168 is added to two 50 ml falcon tubes: one tube receives 200 ug (1 ug/ul) of respective plasmid DNA
169 (for RBD or full-length spike) while the other tube receives 640 ul of ExpiFectamine transfection
170 reagent. The contents of both the 50 ml Falcon tubes are mixed together and incubated at RT for
171 10 minutes after which the transfection mixture is added dropwise to the cells. Cells are then
172 returned to the shaking incubator. Sixteen hours post transfection, 1.2 ml of Expifectamine 293
173 Transfection Enhancer 1 and 12.1 ml of Expifectamine 293 Transfection Enhancer 2 is added to
174 the culture and subsequently, the culture is returned to the shaking incubator.

175 Three days post-transfection, the cells are harvested and spun at 4,000g for 20 minutes at 4°C.
176 The supernatant is filtered using a 0.22 um stericup filter, the cell pellet can be discarded.
177 Alternately, cells can be spun at 200g for 10 minutes, supernatant can be collected, and the same
178 cells can be resuspended in 200 mls of fresh Expi293 expression medium and returned to the
179 shaking incubator for another 3 days. This alternate strategy works well with the RBD but is less
180 suitable for the full-length spike (we have detected protein degradation in that case).

181 Ideally is the supernatant containing the protein is further processed immediately. Alternatively, if
182 it is stored, it must be kept at 4°C (and for no longer than overnight/16h) in order to prevent
183 denaturation of the protein at room temperature.

184

185 **Protein purification via gravity flow:**

186 *Note: This step can be substituted with more advanced purification methodology if e.g. an Aekta*
187 *purifier is available. The methods described below work, even in labs not geared towards protein*
188 *purification.*

189 Prior to use, Ni-NTA resin (6 ml per 200 ml culture) is washed with fresh PBS, then spun at 2000g
190 for 10 min in a centrifuge. Once the centrifugation is complete, PBS is discarded, and resin is
191 resuspended with the supernatant from cells and inverted about two or three times. The resin is
192 then incubated with the supernatant for 2 hours on a shaker at RT.
193
194 Two clean polypropylene columns are loaded with the supernatant-resin mixture and then washed
195 with Wash Buffer four times. Columns are then eluted using the Elution Buffer. Which contains a
196 high concentration of imidazole Four fractions are collected from each column by incubating the
197 resin in the column with 3 ml of Elution Buffer for each fraction. Eluate is collected directly in a 50
198 ml falcon tube placed on ice. The total volume of eluate should be 24 ml from the two columns.
199 More columns can be used to speed up the purification time depending on the volume of the
200 culture.
201
202 Eluate is spun through 10 kDa Amicon Centrifugal Filter Units (for RBD) or 50 kDa Amicon
203 Centrifugal Filter Units (for full-length spike) at 4000g for 30 minutes (or longer if eluate takes
204 longer to pass through the membrane) at 4°C until only 200-300 ul remain in the unit. The

205 Centrifugal Filter Unit is then washed with PBS twice by centrifugation at 4000g for 30 minutes at
206 4°C (washing means filling up with PBS and centrifugation until the volume in the unit is down to
207 200-300ul again). Finally, the protein is collected from the Amicon centrifugal unit, concentration
208 is measured (e.g. using Bradford reagent or similar methods), and a denaturing SDS-page is run
209 to check integrity of the purified protein.
210
211 After the elution step, protein is always kept on ice. For storage longer than 24h it should be frozen
212 to -80°C to avoid degradation.
213

214 **Wash buffer (4L):**

215 NaH_2PO_4. H_2O 31.74 g
216 NaCl   70.16 g
217 Imidazole   5.44 g (final concentration is 20 mM)
218 Distilled water*   4L
219
220

221 **Elution buffer (4L):**
222 NaH_2PO_4. H_2O 31.74 g
223 NaCl   70.16 g
224 Imidazole   64.0 g (final concentration is 235 mM)
225 Distilled water*   4L
226
227 *Use Distilled water filtered using a 0.22um stericup vacuum filtration system.
228
229

230 **Part II: A two-step ELISA protocol for high throughput screening of human serum**
231 **samples for antibodies binding to the spike protein of SARS-CoV-2**
232
233 The purpose of this part of the protocol is to describe the procedure for measuring human antibody
234 responses to the recombinant receptor binding domain (RBD) of the spike protein or full-length
235 spike protein of SARS-CoV-2 and to ensure the reproducibility and consistency of the obtained
236 results.
237 We developed this as a two-step ELISA in which the first step (**A**) includes relatively high
238 throughput screening of samples in a single serum dilution against the RBD (which expresses
239 very well and therefore there is typically more protein available). This is followed by a second step
240 (**B**) in which positive samples from the first step undergo a confirmatory ELISA against the full
241 length spike protein (which is harder to purify, therefore there is usually less available). For the
242 second step a dilution curve is performed. Typically, if only one operator is available, screening
243 ELISAs can be run in the morning (760 samples/10 plates per run) and confirmatory ELISAs can
244 be run in the afternoon (140 samples/10 plates per run). Of note, we describe the assay here as
245 set up in our laboratory. We use a plate washer and a plate reader but no automated system. The
246 protocol can be adapted to an automated liquid handler as well. In addition, one of the difficulties
247 to set up the assay is the availability of appropriate negative and positive controls. Negative
248 controls are easier to come by and can be serum pools of serum taken before 2020. Positive
249 controls can be convalescent samples from COVID19 patients or monoclonal antibodies (mAbs)
250 like CR3022[9,10]. If no human sera or mAbs are available, mouse mAbs, mouse sera against
251 SARS-CoV-2, order animal sera against SARS-CoVo2 or anti-his tag antibodies (the proteins are
252 his-tagged) can be used. However, in this case a different secondary antibody for the species
253 from which the primary antibody is derived is needed for the positive control. Also, we recommend

6

254 generating large batches of positive controls, which can be used for many runs. The positive
255 control should be selected to exceed an OD_{490} of the negative control plus 3 standard deviations
256 of the negative controls up to, at least, a 1:150 dilution. ELISAs can be run with both serum and
257 plasma.
258
259 *Of note: RBD or full length spike might be used for both ELISA steps if only one antigen is*
260 *available. In addition, only step A (not recommended) or only step B might be performed if fewer*
261 *resources are available.*
262
263 - **MATERIALS**
264   - Recombinant RBD protein
265   - Recombinant full-length spike protein
266   - Flat-Bottom Immuno Nonsterile 96-Well Plates 4 HBX (Thermo Scientific #3855,
267   or equivalent)
268   - Flat Bottom Cell Culture Plates (Corning #3599 or equivalent)
269   - Milk Powder (AmericanBio #AB10109-01000, or equivalent)
270   - PBS (1X) (Gibco #10010-023 or equivalent)
271   - Water For Injection (WFI) for Cell Culture (Gibco #A1287301 or equivalent)
272   - Tween 20 (Fisher Bioreagents #BP337-500, or equivalent)
273   - Phosphate Buffered Saline (10X) (Corning™ 46013CM or equivalent)
274   - Polypropylene sterile conical tubes
275   - 15 mL (Denville Scientific #C1018P or equivalent)
276   - 50 mL (Fisher Denville Scientific #C1060P or equivalent)
277   - Sterile, serological pipettes
278   - 5mL (Falcon #356543 or equivalent)
279   - 10mL (Falcon #357551 or equivalent)
280   - 25 mL (Falcon #357535 or equivalent)
281   - 50 mL (Falcon #356550 or equivalent)
282   - Micropipette tips
283   - 20 μL barrier tips (Denville Scientific #P1121 or equivalent)
284   - 200 μL barrier tips (Denville Scientific #P1122 or equivalent)
285   - 200 μL tips (USA Scientific #1111-1700 or equivalent)
286   - 1000 μL barrier tips (Denville Scientific #P1126 or equivalent)
287   - Sterile reservoirs (Fisher Scientific #07-200-127 or equivalent)
288   - Anti-Human IgG (Fab specific)-Peroxidase antibody produced in goat (Sigma
289   #A0293)
290   - Hydrochloric Acid 3.0M (Fisher Scientific #S25856, or equivalent)
291   - SIGMA*FAST*™ OPD (Sigma-Aldrich #P9187 or equivalent)
292   - Kimberly-Clark Kimwipes (Kimberly-Clark Professional #34721 or equivalent)
293   - Pipet-Aid
294   - Micropipettes
295   - Class II biological safety cabinet
296   - Ultra-Low Freezer (-80°C)
297   - Refrigerator at 4°C (+/- 1°C)
298   - Multichannel pipette(s) capable of pipetting 50-250 μL
299   - 1.5 mL Eppendorf tubes (Denville #C2170 or equivalent)
300   - Timer
301   - Aquamax 2000 Plate Washer (Molecular Devices #AQUAMAX 2000 or equivalent)
302   - Biotek SynergyH1 Microplate Reader or equivalent
303
304 - **DEFINITIONS**

305
306 ○ RBD = Receptor Binding Domain
307 ○ ELISA = Enzyme-Linked Immunosorbent Assay
308 ○ PBS = Phosphate-Buffered Saline
309 ○ PBS-T = Phosphate-Buffered Saline with 0.1% Tween 20
310 ○ RT = Room Temperature (18-25°C)
311 ○ HRP = Horseradish Peroxidase
312 ○ HCl = Hydrochloric Acid
313 ○ OPD = o-phenylenediamine dihydrochloride
314
315
316 ● **A - RBD Screening ELISA**
317
318 1. Coating ELISA plates (day 1)
319 ● Thaw the required number of vials of antigen (SARS-CoV-2 **RBD** protein) to coat 96-
320 well microtiter ELISA plates at a concentration of 2 µg/ml. Once thawed, mix by gently
321 vortexing vial before diluting in 1X PBS.
322 ● Prepare approximately 5 mL for each plate to be coated.
323 ● Coat plates with 50 µl of diluted protein per well using a multichannel pipette and a
324 reservoir. Lightly tap plates against surface to ensure protein is evenly coating the
325 bottom of every well.
326 ● Incubate at 4°C overnight. Plates can be stored at 4°C for up to 1 week.
327 ● Always keep a cover plate on top of coated plates during all steps of the protocol!
328
329 2. Heat inactivation of samples (day 1, this is a safety precaution)
330 ● Set the water bath to 56°C. Once temperature is reached, place the serum/plasma
331 samples in and start the timer for 1h immediately.
332 ● Remove samples when the timer goes off. Do not leave samples at 56°C for longer
333 than 1h. Store at 4°C overnight or until use.
334
335 3. Block ELISA plate (day 2)
336 ● Calculate to prepare at least 30 ml of blocking solution per plate.
337 ● Blocking solution consists of PBS-T + 3% milk powder (weight/volume).
338 ● Using an automated plate washer, wash coated ELISA plates 3x with PBS-T.
339 ● Add 200 µl blocking solution to all wells of the plates, starting the timer for 1h (do not
340 exceed 4h) after completing the first plate. Place plates in a 20°C (RT) incubator.
341 *Note: This step (and wherever a plate washer is needed below) can also be performed*
342 *by washing plates with a multichannel pipette by hand if no plate washer is available.*
343
344 4. Pre-diluting samples (day 2)
345 ● In a biological safety cabinet, set up sterile Eppendorf tubes to pre-dilute serum
346 samples 1:5.
347 ● Add 40 µl of sterile 1X PBS to all tubes.
348 ● Gently vortex serum sample to mix and add 10 µl to the Eppendorf tube, vortexing
349 once more. Do this for all remaining samples including the positive and negative
350 controls. *Volume not needed in this part A will be stored and used for part B.*
351
352 5. Dilution plate set-up (day 2)
353 ● Calculate and prepare at least 30 ml of PBS-T + 1% milk powder (weight/volume).

354   ● Prepare 1 dilution plate (separate flat bottomed cell culture plate) per antigen coated
355   plate prepared.
356   ● Add 180 µl of PBS-T containing 1% milk to all wells of the dilution plate (including blank
357   wells)
358   ● Leaving Columns 1 and 12 as blanks, add 20 µl of sample (or control) into the
359   designated well. This results in a final serum dilution of 1:50.
360   ● Continue until all samples and controls have been added to designated wells. See
361   reference plate layout below.
362
363

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	(+) Ctrl.	(-) Ctrl.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Blank
B	Blank	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	...	...	...			Blank
C	Blank											Blank
D	Blank											Blank
E	Blank											Blank
F	Blank											Blank
G	Blank											Blank
H	Blank		...	...	...	Sample 73	Sample 74	Sample 75	Sample 76	(+) Ctrl.	(-) Ctrl.	Blank

364
365
366
367   6. Transfer serum dilution (day 2)
368   ● After blocking incubation, remove plates from the room temperature incubator and
369   throw off the blocking solution. Tap the plates dry on a kimwipe.
370   ● Using a multichannel pipette, pipette up and down 4-6 times in the first row of dilution
371   plate to mix.
372   ● Transfer 100 µl to the corresponding rows in the ELISA plate. Change tips and
373   continue to transfer second row to the ELISA plate.
374   ● Start the timer for 2h as soon as all the rows have been transferred to the first ELISA
375   plate. (Do not exceed 4h)
376   ● Place plates in a 20°C (RT) incubator.
377
378   7. Secondary Antibody (day 2)
379   ● After 2h, wash the plates 3x with PBS-T using the automated plate washer.
380   ● Dilute anti-human IgG (Fab specific) HRP labeled secondary antibody 1:3000 in PBS-
381   T containing 1% milk. Prepare at least 5 ml per plate.

382	●	Add 50 µl to all wells of the plate using a multichannel pipette. Be sure to avoid touching the tips of the pipette to the walls of the well to avoid carry over and high background signals.
383		
384		
385	●	Start the timer for 1h (stay in a range of 50min to 65min) as soon as the secondary antibody has been added to the first plate. Place plates in a 20°C (RT) incubator.
386		
387		
388	8.	Plate development and reading (day 2)
389	●	After 1h, wash plates 3x with PBS-T using an automated plate washer.
390	●	Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1 gold + 1 silver tablet) dissolved in 20 ml WFI can be used for 2 plates.
391		
392	●	Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until ready to start adding to the plates (needs to be prepared fresh right before use).
393		
394	●	Add 100 µl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has been added to the first row on the first plate. Do not exceed 10 minutes of developing before stopping the reaction.
395		
396		
397	●	To stop the reaction after exactly 10 minutes, add 50 µl of 3M HCl to all wells.
398	●	Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after adding HCl) and record data.
399		
400	●	Samples that exceed certain OD_{490} cutoff value (proposed cutoff: OD_{490} = 0.15-0.2 or mean of negative controls plus 3 times the standard deviation of the negative controls) are assigned presumptive positive and will be tested in confirmatory ELISA using full-length spike protein.
401		
402		
403		
404	●	OD_{490} cutoff has to be experimentally determined and depends on assay background and noise.
405		

382   ●   Add 50 µl to all wells of the plate using a multichannel pipette. Be sure to avoid
383   touching the tips of the pipette to the walls of the well to avoid carry over and high
384   background signals.
385   ●   Start the timer for 1h (stay in a range of 50min to 65min) as soon as the secondary
386   antibody has been added to the first plate. Place plates in a 20°C (RT) incubator.
387

388   8.   Plate development and reading (day 2)
389   ●   After 1h, wash plates 3x with PBS-T using an automated plate washer.
390   ●   Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1
391   gold + 1 silver tablet) dissolved in 20 ml WFI can be used for 2 plates.
392   ●   Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until
393   ready to start adding to the plates (needs to be prepared fresh right before use).
394   ●   Add 100 µl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has
395   been added to the first row on the first plate. Do not exceed 10 minutes of developing
396   before stopping the reaction.
397   ●   To stop the reaction after exactly 10 minutes, add 50 µl of 3M HCl to all wells.
398   ●   Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after
399   adding HCl) and record data.
400   ●   Samples that exceed certain OD_{490} cutoff value (proposed cutoff: OD_{490} = 0.15-0.2 or
401   mean of negative controls plus 3 times the standard deviation of the negative controls)
402   are assigned presumptive positive and will be tested in confirmatory ELISA using full-
403   length spike protein.
404   ● OD_{490} cutoff has to be experimentally determined and depends on assay background
405   and noise.
406
407

408   ●   **B - Spike confirmatory ELISA**
409

410   1.   Coating ELISA plates (day 1)
411   ●   Thaw the required number of vials of antigen (SARS-CoV-2 **Spike** protein) to coat 96-
412   well microtiter ELISA plates at a concentration of 2 µg/ml. Once thawed, mix by gently
413   vortexing vial before diluting in 1X PBS.
414   ●   Prepare approximately 5 mL for each plate to be coated.
415   ●   Coat plates with 50 µl of diluted protein per well using a multichannel pipette and a
416   reservoir. Lightly tap plates against surface to ensure protein is evenly coating the
417   bottom of every well.
418   ●   Incubate at 4°C overnight. Plates can likely be stored in 4°C for up to 1 week but this
419   needs to be validate locally to ascertain that it does not change rhe results.
420
421

422   2.   Block ELISA plate (day 2)
423   ●   Calculate to prepare at least 30 ml of blocking solution per plate.
424   ●   Blocking solution consists of PBS-T + 3% milk powder (weight/volume).
425   ●   Using an automated plate washer, wash coated ELISA plates 3x with PBS-T.
426   ●   Add 200 µl blocking solution to all wells of the plates, starting the timer for 1 h (do not
427   exceed 4h) after completing the first plate. Place plates in a 20°C (RT) incubator.
428

429   3.   Pre-diluting samples (day 2)
430   ●   Retrieve 1:5 pre-diluted samples from Part A to be tested and confirmed (samples that
431   are above certain threshold in RBD screening ELISA based on a set OD_{490} value –
432   see end of **A**).

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4. Serial dilution (day 2)
   - Calculate and prepare at least 20 ml of PBS-T + 1% milk powder (weight/volume) per plate.
   - After blocking incubation, remove plates from the room temperature incubator and throw off the blocking solution. Tap the plates dry on a kimwipe.
   - Using a multichannel pipette, add 120 µl of PBS-T containing 1% milk to all wells of the plate.
   - Leaving Columns 1 and 12 as BLANKS, add an extra 51 µl only to Columns 2 and 7 (=sample wells).
   - Add 9 µl of 1:5 pre-diluted sample (final dilution 1:100 on the plate) to the first well in Column 2 and continue to add samples to all 8 wells. In Column 7, add samples to wells 1 through 6. Transfer positive and negative control into wells 7 and 8, respectively. See reference plate layout below.
   - With the multichannel pipette, pipette up and down 4-6 times in Column 2 to mix. Discard these tips. With new tips, transfer 60 µl (3-fold dilution) from Column 2 to Column 3 and pipette up and down once 4-6 times to mix. Repeat this until Column 6; discard 60 µl before Column 7.
   - Taking fresh tips mix Column 7 by pipetting. Repeat the same process of transferring, mixing, and discarding tips from Columns 7-11. Once Column 11 is reached, discard 60 µl.
   - Start timer for 2h (do not exceed 4h) once the first ELISA plate has been serially diluted.
   - Place plates in a 20°C (RT) incubator.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Sample 1						Sample 9					Blank
B	Blank	Sample 2						Sample 10					Blank
C	Blank	Sample 3						Sample 11					Blank
D	Blank	Sample 4						Sample 12					Blank
E	Blank	Sample 5						Sample 13					Blank
F	Blank	Sample 6						Sample 14					Blank
G	Blank	Sample 7						(+) Ctrl.					Blank
H	Blank	Sample 8						(-) Ctrl.					Blank

460
461
462   5. Secondary Antibody (day 2)
463   ● After 2h, wash the plates 3x with PBS-T using the automated plate washer.
464   ● Dilute anti-human IgG (Fab specific) HRP labeled secondary antibody 1:3000 in PBS-
465   T containing 1% milk. Prepare at least 5 ml per plate.
466   ● Add 50 µl to all wells of the plate using a multichannel pipette. Be sure to avoid
467   touching the tips of the pipette to the walls of the well.
468   ● Start the timer for 1 h (50 – 65 min) as soon as the secondary antibody has been
469   added to the first plate. Place plates in a 20°C (RT) incubator.
470
471   6. Plate development and reading (day 2)
472   ● After 1h, wash plates 3x with PBS-T using an automated plate washer.
473   ● Prepare SigmaFast OPD solution and calculate amount needed. One set of tablets (1
474   gold + 1 silver tablet) dissolved in 20 mL WFI can be used for 2 plates.
475   ● Fully dissolve one gold tablet in 20 mL WFI. Do not add silver tablet to solution until
476   ready to start adding to the plates.
477   ● Add 100 µl to all wells of the plate. Begin timer for 10 minutes as soon as OPD has
478   been added to the first row of the first plate. Do not exceed 10 minutes of developing
479   before stopping the reaction.
480   ● To stop the reaction after exactly 10 minutes, add 50 µl of 3M HCl to all wells.
481   ● Read ELISA plates in plate reader at an absorbance of 490 nm (immediately after
482   adding HCl) and record data.
483   ● True positive samples will have a signal higher than the negative control plus 3
484   standard deviations of the negative controls in at least two consecutive dilutions.
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496
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498
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521

Prolonged presence of SARS-CoV-2 viral RNA in faecal samples

We present severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR results of all respiratory and faecal samples from patients with coronavirus disease 2019 (COVID-19) at the Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, China, throughout the course of their illness and obligated quarantine period. Real-time RT-PCR was used to detect COVID-19 following the recommended protocol (appendix p 1). Patients with suspected SARS-CoV-2 were confirmed after two sequential positive respiratory tract sample results. Respiratory and faecal samples were collected every 1–2 days (depending on the availability of faecal samples) until two sequential negative results were obtained. We reviewed patients' demographic information, underlying diseases, clinical indices, and treatments from their official medical records. The study

was approved by the Medical Ethical Committee of The Fifth Affiliated Hospital of Sun Yat-sen University (approval number K162-1) and informed consent was obtained from participants. Notably, patients who met discharge criteria were allowed to stay in hospital for extended observation and health care.

Between Jan 16 and March 15, 2020, we enrolled 98 patients. Both respiratory and faecal samples were collected from 74 (76%) patients. Faecal samples from 33 (45%) of 74 patients were negative for SARS CoV-2 RNA, while their respiratory swabs remained positive for a mean of 15·4 days (SD 6·7) from first symptom onset. Of the 41 (55%) of 74 patients with faecal samples that were positive for SARS-CoV-2 RNA, respiratory samples remained positive for SARS-CoV-2 RNA for a mean of 16·7 days (SD 6·7) and faecal samples remained positive for a mean of 27·9 days (10·7) after first symptom onset (ie, for a mean of 11·2 days [9·2] longer than for respiratory samples). The full disease course of the 41 patients with faecal

samples that were positive for SARS-CoV-2 RNA is shown in the figure. Notably, patient 1 had positive faecal samples for 33 days continuously after the respiratory samples became negative, and patient 4 tested positive for SARS-CoV-2 RNA in their faecal sample for 47 days after first symptom onset (appendix pp 4–5).

A summary of clinical symptoms and medical treatments is shown in the appendix (pp 2–3, 6–8). The presence of gastrointestinal symptoms was not associated with faecal sample viral RNA positivity (p=0·45); disease severity was not associated with extended duration of faecal sample viral RNA positivity (p=0·60); however, antiviral treatment was positively associated with the presence of viral RNA in faecal samples (p=0·025; appendix pp 2–3). These associations should be interpreted with caution because of the possibility of confounding. Additionally, the Ct values of all three targeted genes (RdRp, N, E) in the first faecal sample that was positive for viral RNA were negatively associated with the duration of faecal viral RNA positivity (RdRp gene r =−0·34; N gene
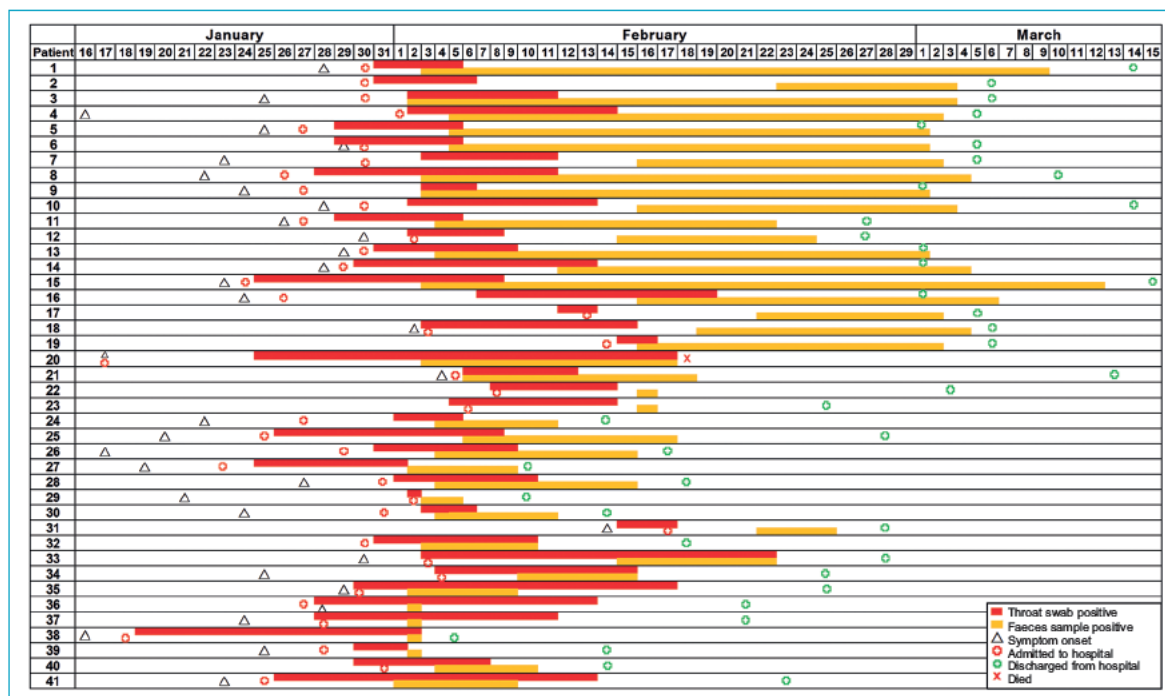
Figure: Timeline of results from throat swabs and faecal samples through the course of disease for 41 patients with SARS-CoV-2 RNA positive faecal samples, January to March, 2020

$r = -0.02$; and E gene $r = -0.16$), whereas the correlation of the Ct values with duration of faecal sample positivity was only significant for $RdRp$ (p=0·033; N gene p=0·91; E gene p=0·33).

Our data suggest the possibility of extended duration of viral shedding in faeces, for nearly 5 weeks after the patients' respiratory samples tested negative for SARS-CoV-2 RNA. Although knowledge about the viability of SARS-CoV-2 is limited,[1] the virus could remain viable in the environment for days, which could lead to faecal–oral transmission, as seen with severe acute respiratory virus CoV and Middle East respiratory syndrome CoV.[2] Therefore, routine stool sample testing with real-time RT-PCR is highly recommended after the clearance of viral RNA in a patient's respiratory samples. Strict precautions to prevent transmission should be taken for patients who are in hospital or self-quarantined if their faecal samples test positive.

As with any new infectious disease, case definition evolves rapidly as knowledge of the disease accrues. Our data suggest that faecal sample positivity for SARS-CoV-2 RNA normally lags behind that of respiratory tract samples; therefore, we do not suggest the addition of testing of faecal samples to the existing diagnostic procedures for COVID-19. However, the decision on when to discontinue precautions to prevent transmission in patients who have recovered from COVID-19 is crucial for management of medical resources. We would suggest the addition of faecal testing for SARS-CoV-2.[3] Presently, the decision to discharge a patient is made if they show no relevant symptoms and at least two sequential negative results by real-time RT-PCR of sputum or respiratory tract samples collected more than 24 h apart. Here, we observed that for over half of patients, their faecal samples remained positive for SARS-CoV-2 RNA for a mean of 11·2 days after respiratory tract samples became negative for SARS-CoV-2 RNA, implying that the virus is actively replicating in the patient's gastrointestinal tract and that faecal–oral transmission could occur after viral clearance in the respiratory tract.

Determining whether a virus is viable using nucleic acid detection is difficult; further research using fresh stool samples at later timepoints in patients with extended duration of faecal sample positivity is required to define transmission potential. Additionally, we found patients normally had no or very mild symptoms after respiratory tract sample results became negative (data not shown); however, asymptomatic transmission has been reported.[4] No cases of transmission via the faecal–oral route have yet been reported for SARS-CoV-2, which might suggest that infection via this route is unlikely in quarantine facilities, in hospital, or while under self-isolation. However, potential faecal–oral transmission might pose an increased risk in contained living premises such as hostels, dormitories, trains, buses, and cruise ships.

Respiratory transmission is still the primary route for SARS-CoV-2 and evidence is not yet sufficient to develop practical measures for the group of patients with negative respiratory tract sample results but positive faecal samples. Further research into the viability and infectivity of SARS-CoV-2 in faeces is required.
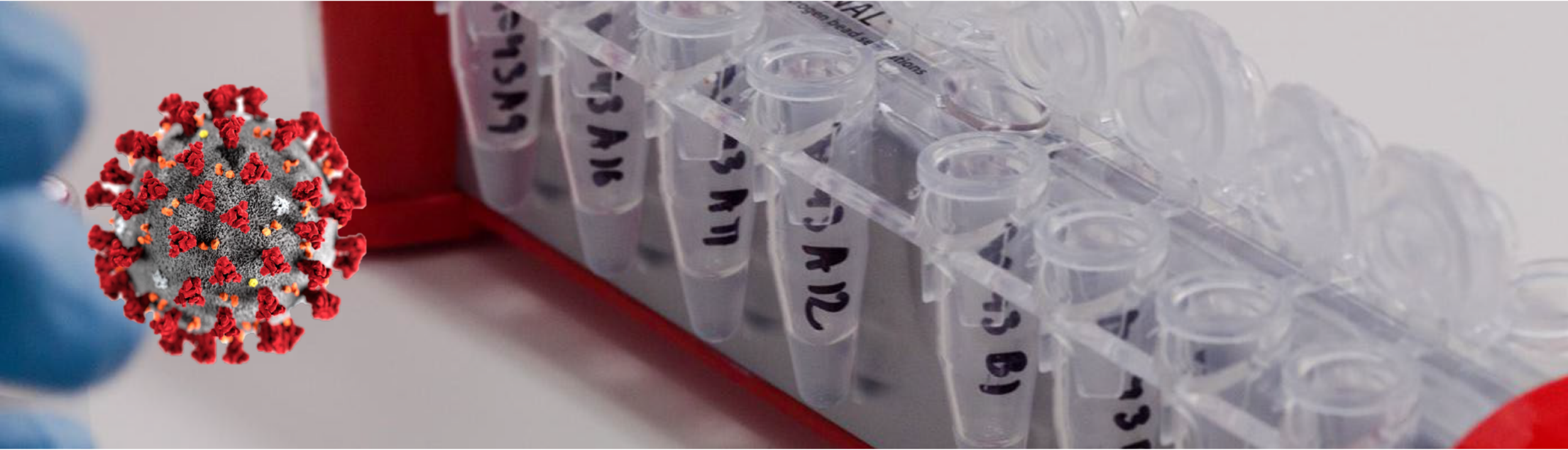
Yongjian Wu, Cheng Guo, Lantian Tang, Zhongsi Hong, Jianhui Zhou, Xin Dong, Huan Yin, Qiang Xiao, Yanping Tang, Xiujuan Qu, Liangjian Kuang, Xiaomin Fang, Nischay Mishra, Jiahai Lu, Hong Shan, Guanmin Jiang, Xi Huang
huangxi1312@163.com

Center for Infection and Immunity, Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, 519000, China (YW, LT, ZH, JZ, XD, HY, QX, YT, XQ, LK, XF, GJ, XH); Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY, USA (CG, NM); School of Public Health, Sun Yat-sen University, Guangzhou, Guangdong Province, China (JL); and Guangdong Provincial Engineering Research Center of Molecular Imaging, Guangdong Provincial Key Laboratory of Biomedical Imaging, and Department of Interventional Medicine, Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China (HS)

1   Goh GK-M, Dunker AK, Foster JA, Uversky VN. Rigidity of the outer shell predicted by a protein intrinsic disorder model sheds light on the COVID-19 (Wuhan-2019-nCoV) infectivity. *Biomolecules* 2020; **10:** e331.

2   Yeo C, Kaushal S, Yeo D. Enteric involvement of coronaviruses: is faecal–oral transmission of SARS-CoV-2 possible? *Lancet Gastroenterol Hepatol* 2020; published online Feb 19. https://doi.org/10.1016/S2468-1253(20)30048-0.

3   Munster VJ, Koopmans M, van Doremalen N, van Riel D, de Wit E. A novel coronavirus emerging in China - key questions for impact assessment. *N Engl J Med* 2020; **382:** 692–94.

4   Bai Y, Yao L, Wei T, et al. Presumed asymptomatic carrier transmission of COVID-19. *JAMA* 2020; published online Feb 21. DOI:10.1001/jama.2020.2565.

EVALUATING THE SEROPREVALENCE OF SARS-COV-2 ANTIBODIES IN AN ASYMPTOMATIC POPULATION AT MD ANDERSON CANCER CENTER

PROJECT ARC🦠 (Antibody **R**esponse in **CO**VID19**)**

Lead and co-lead: Nadim Ajami and Eleonora Dondossola
MDACC Collaborators: Giannicola Genovese, Jennifer Wargo, Pam Sharma,
External Collaborators: Tony Piedra (BCM), Maria Elena Bottazzi (BCM), Florian Krammer (Mount Sinai), Michael Laposata (UTMB)
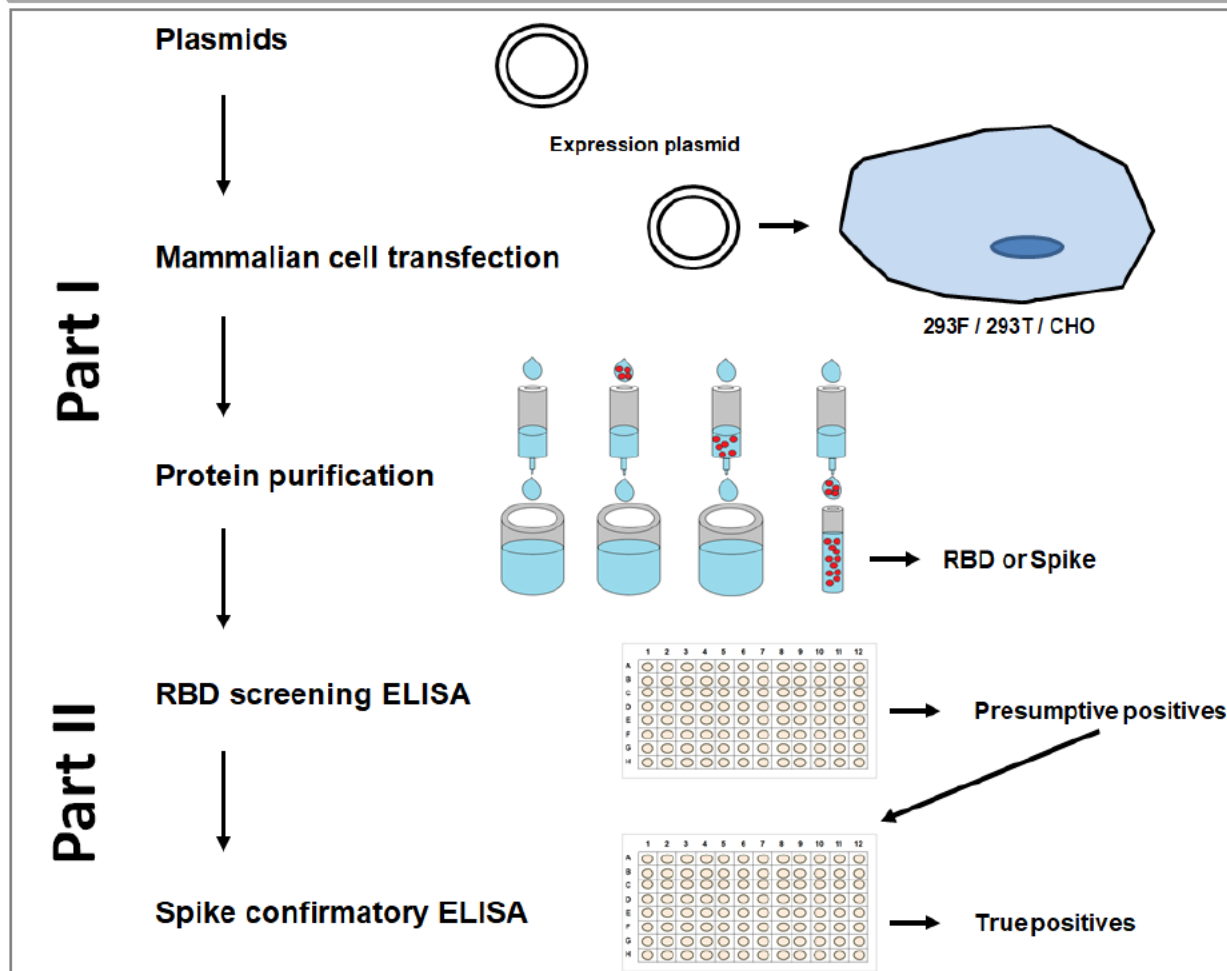
(UNIVERSAL) REAGENTS

Antigens and Antibodies:

- **Receptor Binding Domain of SARS-CoV-2 (NR-52306)**
    - Plasmid grown and RBD purified in-house
    - Antigen ordered from BEI Resources (ordered, limited quantity)
    - Antigen sourced from BCM (Maria Elena Bottazzi, MTA wip)

- **Spike protein of SARS-CoV-2 (NR-52306)**
    - Plasmid grown and RBD purified in-house (ramping-up)
    - Antigen ordered from BEI Resources (ordered, limited quantity)

- **Goat Anti-Human IgG HRP (Sigma)**
- **Goat Anti-Human IgM HRP (Sigma)**
- **Rabbit SARS-CoV-2 S1 mAb (Sino Biological)**

ASSAY SETUP

PLASMID EXPRESSION, PROTEIN PURIFICATION, ASSAY SETUP

Part I

Plasmids

Expression plasmid

Mammalian cell transfection

293F / 293T / CHO

Protein purification → RBD or Spike

Part II

RBD screening ELISA → Presumptive positives

Spike confirmatory ELISA → True positives

RBD-SARS-CoV-2
- BEI Resources
- Maria Elena Bottazzi (BCM)
- Eleonora Dondossola (MDACC)
- Gianni Genovese (MDACC)

SPIKE-SARS-CoV-2
- BEI Resources
- Gianni Genovese (MDACC)
- Eleonora Dondossola (MDACC)

POSITIVE SERA
- ITB MDACC
- Pathology, UTMB
- Tony Piedra, BCM

PREPANDEMIC SERA
- Jen Wargo
- Tony Piedra, BCM
- Pathology, UTMB

Project ARC | May 1st, 2020

ASSAY UTILIZATION

SCREEN ELISA (RBD)

Day1

- Precoated and blocked plate
- Heat inactivate samples
- Predilute samples
- Sample dilution
- Secondary Ab
- Develop

Throughput: 1 operator, 760 samples/10 plates per run

CONFIRMATORY ELISA (SPIKE)

Day1

- Precoated and blocked plate
- Block
- Predilute samples
- Serial dilutions
- Secondary Ab
- Develop

1 operator, 140 samples/10 plates per run

TIMELINE & ACTIVITES

Week	Apr 27	May 4	May 11	May 18	May 25	June 1	June 8
REAGENTS SOURCING	██	██	██				
ELISA SETUP		██	██	██	██		
ELISA VALIDATION					██	██	
TESTING							██

LAB	ACTIVITY
JEN WARGO (MDACC)	Plasmid amplification & protein expression (RBD and spike)
ELEONORA DONDOSSOLA (MDACC)	Protein purification & ELISA Setup
GIANNI GENOVESE (MDACC)	Plasmid amplification (RBD and spike)
MARIA ELENA BOTTAZZI (BCM)	RBD
TONY PIEDRA (BCM)	Protocol details
MICHAEL LAPOSATA (UTMB)	Positive and pre-pandemic sera

COVID19 SEROPREVALENCE RESEARCH STUDY

PHASE I	PHASE II	PHASE III
ASSAY DEVELOPMENT *4 weeks*	**ASSAY UTILIZATION** *Start June 8th*	**ASSAY EXPANSION** *To be determined*

PHASE I — ASSAY DEVELOPMENT (4 weeks)

MDA – ELISA

- Quantitative
- Antigen: Receptor Binding Domain (screening), and Spike protein (confirmatory)
- Antibody: IgG and IgM
- Controls: Positive sera and mAb; pre-pandemic sera

PHASE II — ASSAY UTILIZATION (Start June 8th)

SET 1

COVID19 confirmed and probable cases – samples banked at ITB

SET 2

Research, healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

COVID19 ELISA

→ **Positive** → Presumed Immune

↓ **Negative** → Not presumed immune

IgM + ; IgG -	Resolving infection
IgM + ; IgG +	Convalescent
IgM - ; IgG +	Recovered

Project ARC | May 1st, 2020

PHASE III — ASSAY EXPANSION (To be determined)

- Expansion to wider population
- Repeat testing to determine duration of antibodies in circulation
- Development of receptor-binding inhibition assays
- Development of neutralization assays (UTMB)

**Tony Piedra, Xunyan Ye, Baylor
College of Medicine**

		Florian Krammer	
		FK RBD-1	FK RBD-2
mAb (SinoBiological, 40150R007)	Rabbit CoV-1-S1	Reactive	Reactive
polyclonal	Rabbit CoV-2	Reactive	Reactive
polyclonal	Control Sera	Negative	Negative

RayBiotech	
CoV-2-S1 subunit	CoV-2 RBD
Negative	Negative
Negative	Negative
Negative	Negative

THE UNIVERSITY OF TEXAS
MD Anderson
Cancer Center

Making Cancer History®

EVALUATING THE SEROPREVALENCE OF SARS-COV-2 ANTIBODIES IN AN ASYMPTOMATIC OR SUBCLINICAL POPULATION AT MD ANDERSON CANCER CENTER

RESEARCH PROTOCOL

Study Chair: Nadim Ajami
Co-chairs: Eleonora Dondossola
Internal Collaborators: Padmanee Sharma, Jennifer Wargo, Alex Lazar
External Collaborators: Tony Piedra (BCM), Florian Krammer (Mount Sinai)

State of affairs

- 5.8k cases (Houston/Harris)
  - ~90 deaths (1.6% CFR)
- 8.9k cases (Houston-area)
  - ~180 deaths (2% CFR)

- FDA-EUA serologic tests
  - DiaSorin Inc (IgG; S1/S2)
  - Ortho-Clinical Diagnostics (IgG, and total Ig)
  - Autobio Diagnostics (rapid test; IgM/IgG)
  - **Mount Sinai (IgG)**
  - Chembio Diagnostic System
  - Cellex (IgM, IgG)
  - False negative results are more frequent around time of infection
  - IgM detection shows higher variability than IgG; best results by measuring total Ig

- BCM – validating assay, testing starts this month
- Methodist – started collecting samples from employees.
- Memorial Hermann – pilot project. Projected to start this week.

http://publichealth.harriscountytx.gov/Resources/2019-Novel-Coronavirus

Quadrant Chart

NE	1.1k
NW	1.8k
SE	725
SW	2.1k
Unknown	85

Combined Age Groups

Harris County & Houston

Research Proposal

The primary aim of this research study is to determine the seroprevalence of antibodies against SARS-CoV-2, the causing agent of COVID19, among our patient population and workforce.

Outcome Measures:

Primary:

- Determine the extent of SARS-CoV-2 infection in our community by means of seropositivity.

Secondary:

- Determine the cumulative incidence of infection in our patient population and workforce.
- Determine the isotype of antibodies raised against surface antigens of SARS-CoV-2

Samples:

- Residual samples ITB, APOLLO

- Workforce: prospectively collected samples, new protocol

Seroconversion and Neutralizing Abs

Viral load kinetics, seroconversion, and clinical observations in individual cases. seroconversion (IgG and IgM) in SARS-CoV-2 patients has been shown to occur by 7 days in 50% of individuals and 14 days in all of them.

Neutralizing antibodies in >95% of patients positive to CoV-2 using a neutralization assay

Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020.

Okba NMA MM, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe Acute Respiratory Syndrome Coronavirus 2−Specific Antibody Responses in Coronavirus Disease 2019 Patients: Centers for Disease Control and Prevention, 2020.

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- Memorial Hermann – pilot project. Projected to start this week.

http://publichealth.harriscountytx.gov/Resources/2019-Novel-Coronavirus

Cross Functional Team – Institutional Effort

via Jason Bock,
Epidemiology Study of MD Anderson Research Staff to Determine Prevalence of Exposure to SAR2-Cov2

- Lab
  - Wargo/Ajami/Dondossola: setting up collaboration with BCM for CoV2 ELISA
  - Kalluri: COVID protein production/ELISA development for whole spike and characterization
    - Collaboration with Yee on neutralization assay
    - Collaboration with Shpall on convalescent plasma
    - Collaboration with ORBIT on humoral response characterization
- Study design and data analysis
  - Paul Scheet, Sonia Cunningham—Epidemiology
- Viral PCR correlative testing
  - James Kelly, Ignacio Wistuba--MDL
- Sample Collection
  - TBD

Research Proposal

The primary aim of this research study is to determine the seroprevalence of antibodies against SARS-CoV-2, the causing agent of COVID19, among our patient population and workforce.

Outcome Measures:

Primary:

- Determine the extent of SARS-CoV-2 infection in our community by means of seropositivity.

Secondary:

- Determine the cumulative incidence of infection in our patient population and workforce.
- Determine the isotype of antibodies raised against surface antigens of SARS-CoV-2

Inclusion:

- Adults >18 yo.
- COVID19 confirmed and probable cases. Residual samples ITB, APOLLO
- Workforce: prospectively collected samples under the seroprevalence protocol.
- 5,000 individuals from 4/1/2020 to 4/1/2021

Seroconversion and Neutralizing Abs

Viral load kinetics, seroconversion, and clinical observations in individual cases. seroconversion (IgG and IgM) in SARS-CoV-2 patients has been shown to occur by 7 days in 50% of individuals and 14 days in all of them.

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COVID19 SEROPREVALENCE RESEARCH STUDY

PHASE I

PHASE II

PHASE III

ASSAY DEVELOPMENT
4 weeks

ASSAY UTILIZATION
Start in 4 weeks, in course as permitted

ASSAY EXPANSION
To be determined

BCM/MDA – ELISA
- Quantitative
- Antigen: Receptor Binding Domain (screening), and Spike protein (confirmatory)
- Antibody: IgG and IgM
- Controls: Positive sera and mAb/polyclonal sera

SET 1

COVID19 confirmed and probable cases – samples banked at ITB

SET 2

Research, healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

COVID19 ELISA

Positive → Presumed Immune

Negative

Not presumed immune

IgM + ; IgG -   Resolving infection

IgM + ; IgG +   Convalescent

IgM - ; IgG +   Recovered

- Expansion to wider population

- Repeat testing to determine duration of antibodies in circulation

- Development of neutralization assays

COVID19 ANTIBODY RAPID SCREENING ALGORITHM FOR PATIENTS, HEALTHCARE AND ESSENTIAL WORKERS INCLUDING THOSE IN HIGH-RISK AREAS NOT PRESENTING SYMPTOMS OF DISEASE

PRIORITY LEVEL 1

Healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

PRIORITY LEVEL 1

Patients recovered from COVID19

PRIORITY LEVEL 2

Patient-facing HCWs not presenting symptoms

PRIORITY LEVEL 3

Essential workers and others in high-risk areas not presenting symptoms

PRIORITY LEVEL 3

Blood products

Serology → **Negative** → Not presumed immune

Retest in 45 days in the absence of symptoms (N/A for blood products)

Positive

RT-qPCR (MolDx Lab) On nasopharyngeal swabs → **Negative** → Serology → **Negative** → Retest in 21 days → **Negative** → Not presumed immune

If prior serology test was positive

Positive

Isolation

Retest in 14 days

Serology **Positive** → Presumed Immune

Retest in 21 days **Positive** → Presumed Immune

IN-HOUSE TESTING: PRIORITY LEVELS 1 & 2
SEND-OUT: PRIORITY LEVELS 2 & 3

COVID19 ANTIBODY RAPID SCREENING ALGORITHM FOR PATIENTS, HEALTHCARE AND ESSENTIAL WORKERS INCLUDING THOSE IN HIGH-RISK AREAS NOT PRESENTING SYMPTOMS OF DISEASE

COVID19 ANTIBODY RAPID SCREENING ALGORITHM FOR HEALTHCARE AND ESSENTIAL WORKERS INCLUDING THOSE IN HIGH-RISK AREAS NOT PRESENTING SYMPTOMS OF DISEASE

SET 1

Research, healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

VALIDATION SAMPLES

Residual samples from patients recovered from COVID19

PRIORITY LEVEL 2

Patient-facing HCWs not presenting symptoms

PRIORITY LEVEL 3

Essential workers and others in high-risk areas not presenting symptoms

PRIORITY LEVEL 3

Blood products

Serology → **Negative** → Not presumed immune → Retest in 45 days in the absence of symptoms (N/A for blood products)

Positive

RT-qPCR (MolDx Lab) → **Negative** → Serology → **Negative** → Retest in 21 days → **Negative** → Not presumed immune

Positive

Isolation

Retest in 14 days

If prior serology test was positive

Positive

Presumed Immune

Positive

SEROLOGICAL EPIDEMIOLOGY FOR COVID19 AT MD ANDERSON CANCER CENTER

PHASE I

ASSAY DEVELOPMENT

BCM/MDA – ELISA
- Quantitative
- Antigen: Spike (full length) and RBD
- Antibody: IgG and IgM

MDA – ELISA
- Quantitative
- Antigen: Spike protein (full-length)
- Antibody: IgG and IgM

PHASE II

ASSAY UTILIZATION

SET 1

500 Research, healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

VALIDATION SAMPLES

~10 Residual samples from patients recovered from COVID19

COVID19 ELISA → **Positive** → Presumed Immune

Negative ↓

Not presumed immune

IgM + ; IgG - Exposed, acute infection

IgM - ; IgG + Exposed, convalescent

IgM + ; IgG + Transition between acute & convalescent phase

PHASE III

ASSAY EXPANSION

- Expansion to wider population

- Repeat testing to determine duration of antibodies in circulation

- Development of neutralization assays

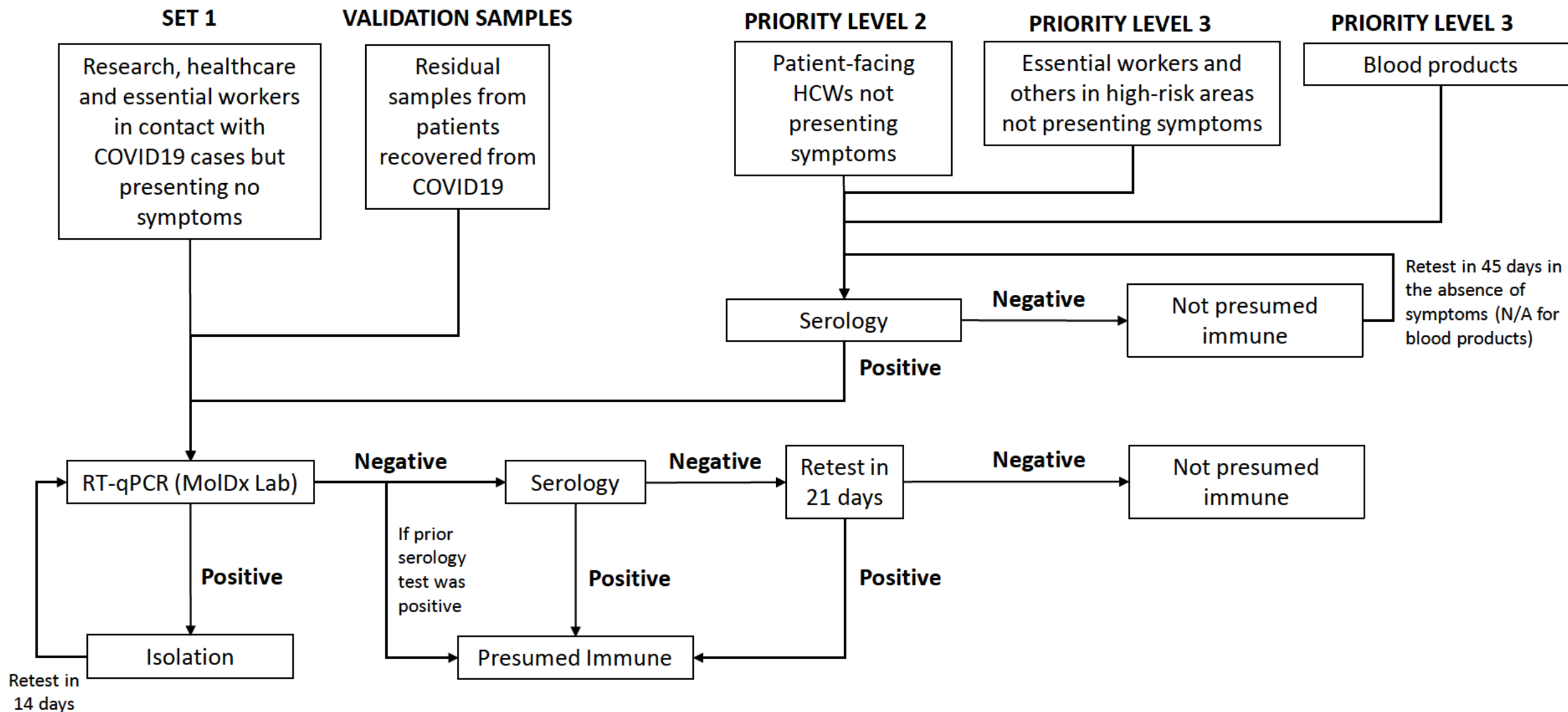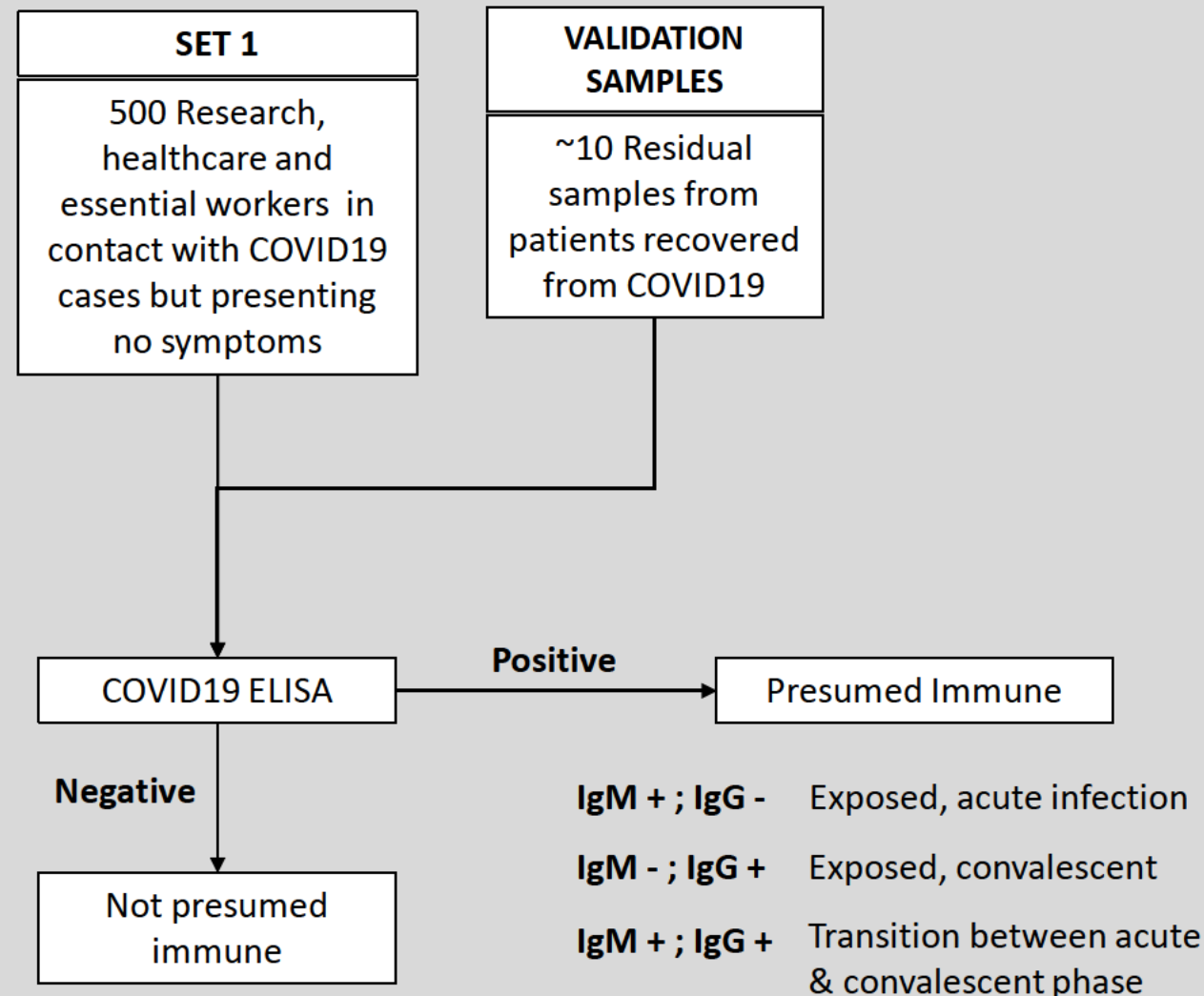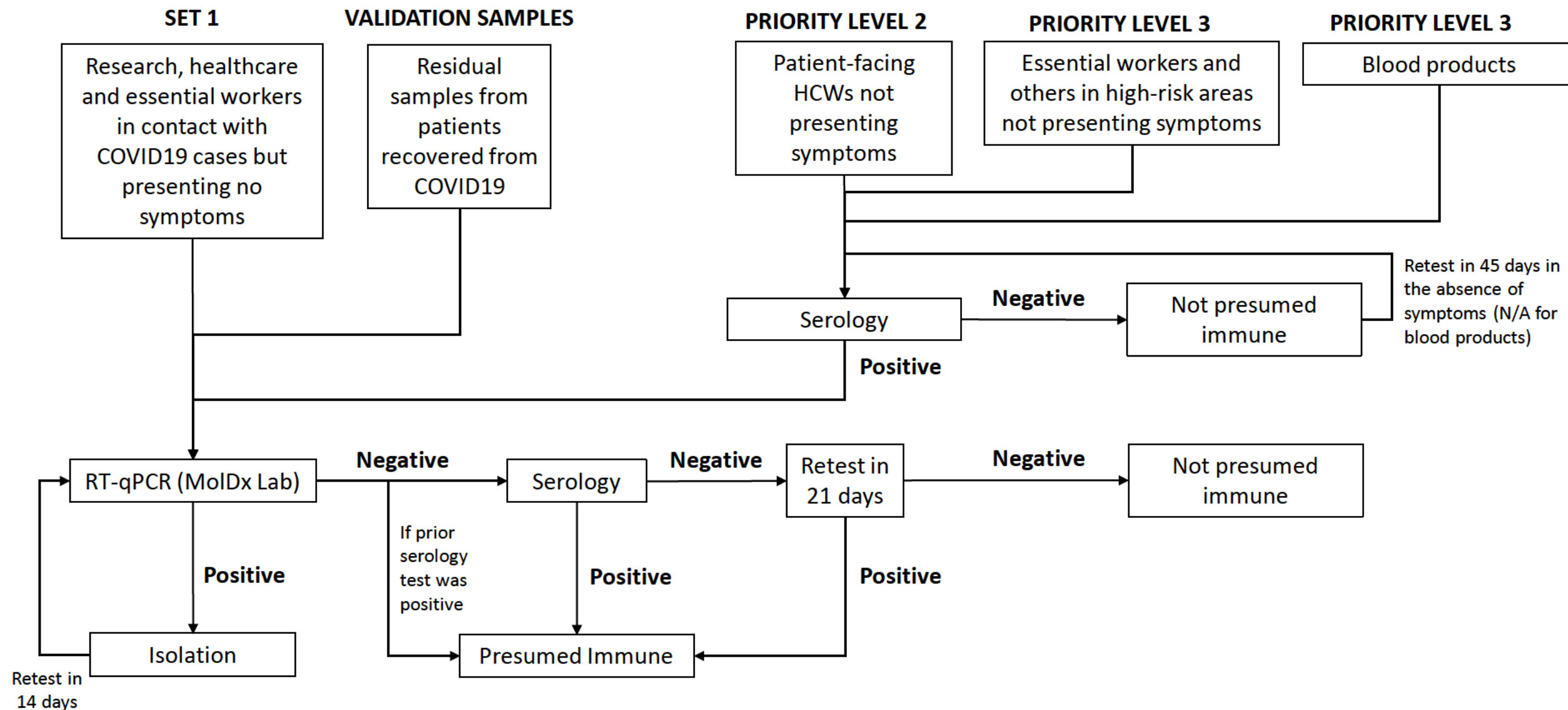# COVID19 ANTIBODY RAPID SCREENING ALGORITHM FOR HEALTHCARE AND ESSENTIAL WORKERS INCLUDING THOSE IN HIGH-RISK AREAS NOT PRESENTING SYMPTOMS OF DISEASE

SEROLOGICAL EPIDEMIOLOGY FOR COVID19 AT MD ANDERSON CANCER CENTER

PHASE I

PHASE II

PHASE III

ASSAY DEVELOPMENT

ASSAY UTILIZATION

ASSAY EXPANSION
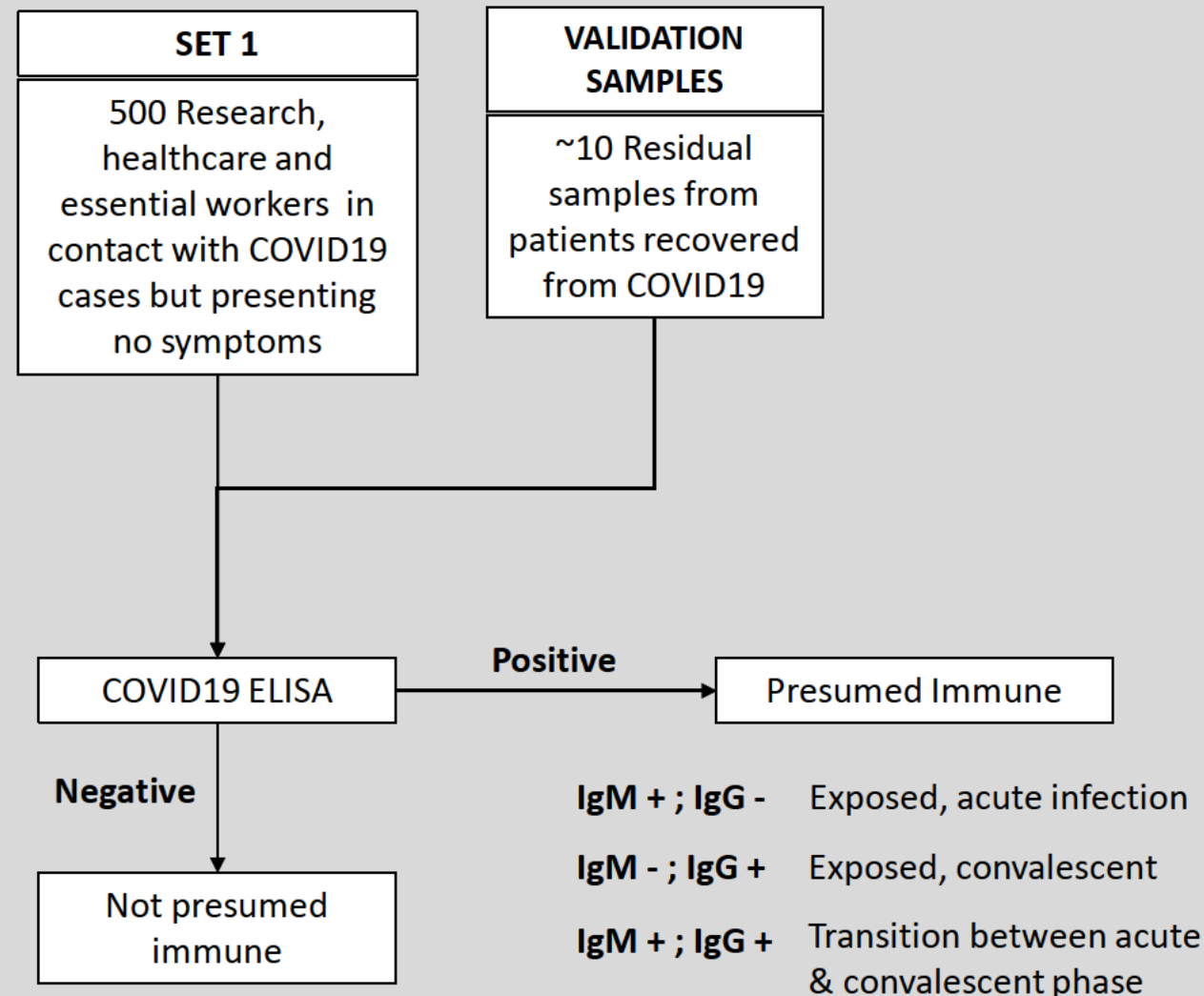
BCM/MDA – ELISA
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- Expansion to wider population
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SEROLOGICAL EPIDEMIOLOGY FOR COVID19 AT MD ANDERSON CANCER CENTER

PHASE I	PHASE II	PHASE III

PHASE I

ASSAY DEVELOPMENT
4 weeks

BCM/MDA – ELISA
- Quantitative
- Antigen: Receptor Binding Domain (RBD)
- Antibody: IgG and IgM
- Controls: Positive sera and mAb/polyclonal sera

MDA – ELISA
- Quantitative
- Antigen: Spike protein (full-length)
- Antibody: IgG and IgM
- Controls: Positive sera and mAb/polyclonal sera

PHASE II

ASSAY UTILIZATION
Start in 4 weeks, in course as permitted

ASSAY SELECTION

One assay will be selected based on sensitivity, specificity, and ease of implementation.

SET 1

500 Research, healthcare and essential workers in contact with COVID19 cases but presenting no symptoms

VALIDATION SAMPLES

~10 Residual samples from patients recovered from COVID19

COVID19 ELISA → **Positive** → Presumed Immune

Negative → Not presumed immune

IgM + ; IgG -   Resolving infection

IgM + ; IgG +   Convalescent

IgM - ; IgG +   Recovered

PHASE III

ASSAY EXPANSION
To be determined

- Expansion to wider population

- Repeat testing to determine duration of antibodies in circulation

- Development of neutralization assays

SEROLOGICAL EPIDEMIOLOGY FOR COVID19 AT MD ANDERSON CANCER CENTER

PHASE I	PHASE II	PHASE III

PHASE I

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4 weeks

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- Antigen: Spike protein (full-length)
- Antibody: IgG and IgM
- Controls: Positive sera and mAb/polyclonal sera

PHASE II

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Start in 4 weeks, in course as permitted
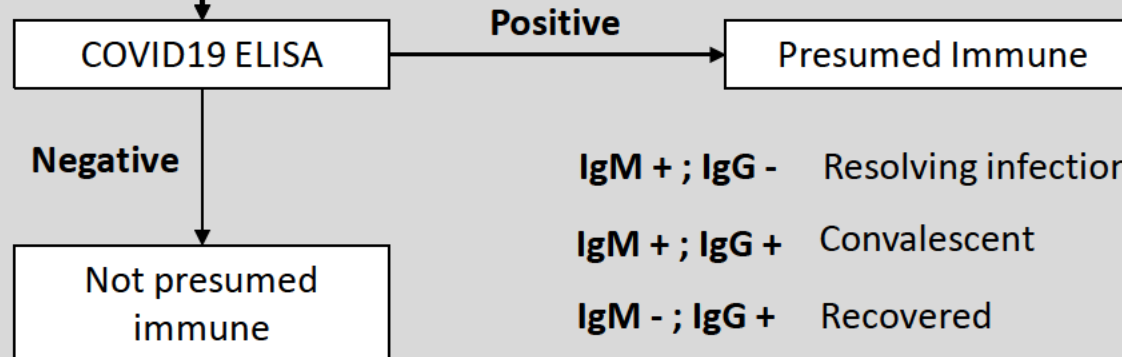
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PHASE III

ASSAY EXPANSION
To be determined

- Expansion to wider population

- Repeat testing to determine duration of antibodies in circulation

- Development of neutralization assays

SIGNIFICANT ALTERATIONS IN FECAL MICROBIOMES IN PATIENTS WITH COVID-19 COMPARED TO CONTROLS

Enrichment of opportunistic pathogens and depletion of beneficial commensals, at time of hospitalization and at all timepoints during hospitalization.
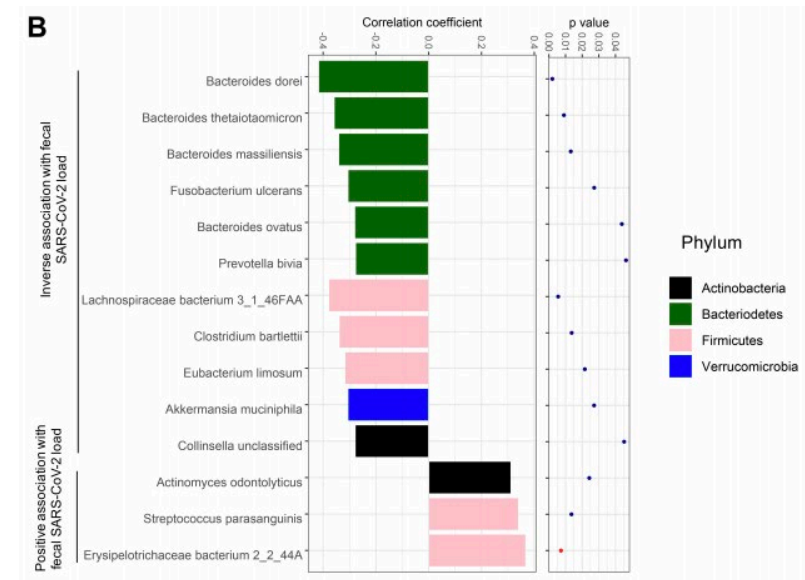
Shotgun metagenomic sequencing analyses of fecal samples (2-3 times per week) from 15 patients with COVID-19 in Hong Kong, from February 5 through March 17, 2020.

Disease categorized as mild (no radiographic evidence of pneumonia), moderate (pneumonia was present), severe (respiratory rate ≥30/min, or oxygen saturation ≤93% when breathing ambient air), or critical (respiratory failure requiring mechanical ventilation, shock, or organ failure requiring intensive care).

Microbiome data with those from 6 subjects with community-acquired pneumonia and 15 healthy individuals (controls)

Adapted from Zao et al. Gastroenterology, May 2020
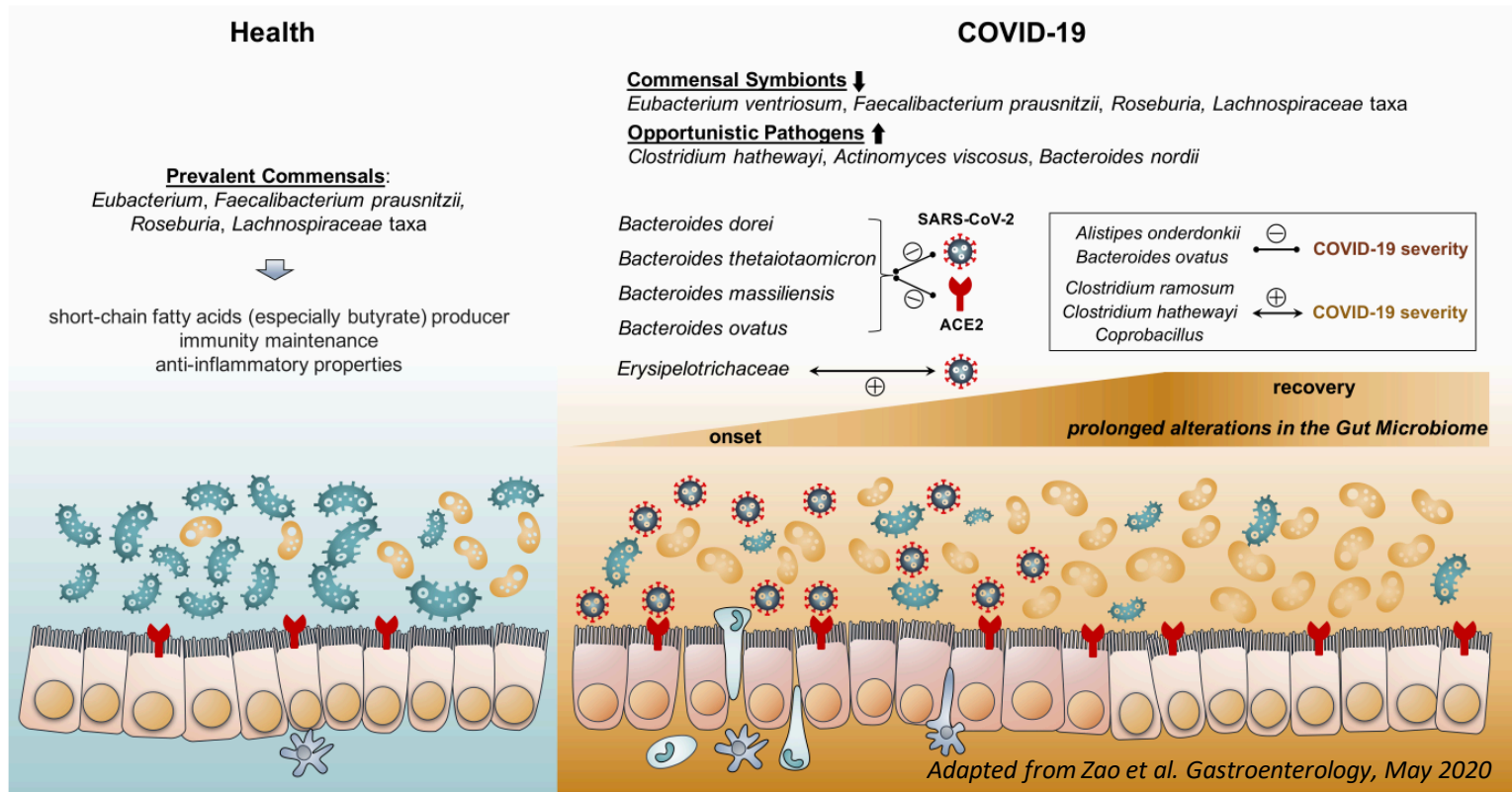
From:	Dianna Hundl
Sent on:	Sunday, March 22, 2020 9 52 16 PM
To:	Najami@mdanderson.org
Subject:	Lyra SARS CoV 2 Assay
Attachments:	image0.jpeg (35.4 KB), ATT00001.htm (312 Bytes), EUA200016 Lyra SARS CoV 2 Assay PI 17Mar2020 final pdf (1.32 MB), ATT00002.htm (333 Bytes), 2-EUA200016 Quidel LOA 03172020 pdf (233 72 KB), ATT00003 htm (337 Bytes), 3 EUA200016 Quidel HCP FS 03172020.pdf (247.23 KB), ATT00004 htm (333 Bytes), 4 EUA200016 Quidel Patient FS 03172020.pdf (195.62 KB), ATT00005.htm (4.68 KB)

WARNING: This email originated from outside of MD Anderson. Please validate the sender's email address before clicking on links or attachments as they may not be safe.

Dr.Ajami,

Here are some of the documents that you can review.

Lyra SARS-COV-2 will be on real-time PCR platform. This is a solution for high throughput, high quality molecular testing to detect and identify infectious diseases.

You need two instrument to run our assay, that are common for labs to have.

It's been validated on; Applied Biosystems$^®$ 7500 Fast Dx Real-Time PCR Instrument, Cepheid$^®$ SmartCycler$^®$ II and Life Technologies QuantStudio^{TM} Dx Real-Time PCR Instrument.

Do you currently have an extraction method?

It was validated on *bioMérieux NucliSENS® ea yMAG® y tem or EMAG®* system or you can do manual extraction.

The kits are available with reagents alone or with swabs/Viral Transport Media included.

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